WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Tuesday, June 15, 2004

Hide?	Set Name	Query	Hit Count
		DB=PGPB; THES=ASSIGNEE; PLUR=YES; OP=ADJ	
☐	L13	L6 and crystal and cysteine substitution	0
		DB=USPT,USOC,EPAB,JPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ	
☐	L12	L6 and crystal and cysteine substitution	0
☐	L11	L6 and crystal and cysteine adj20 muta$10	0
☐	L10	L6 and crystal same cysteine adj20 muta$10	0
		DB=PGPB; THES=ASSIGNEE; PLUR=YES; OP=ADJ	
☐	L9	L6 and crystal same cysteine adj20 muta$10	0
☐	L8	L6 and crustal and cysteine adj20 muta$10	0
☐	L7	L6 and crustal same cysteine adj20 muta$10	0
☐	L6	amyloid adj3 peptide adj1 binding protein or ERAB or hydroxyacyl-coa dehydrogenase or hadha2	38
☐	L5	crystal? and cystine adj20 muta$10	8
☐	L4	crystal? and selenocystine	0
		DB=USPT,USOC,EPAB,JPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ	
☐	L3	crystal? and selenocystine	12
☐	L2	crystal? and selinocystine	0
☐	L1	crystal? and cystine adj20 muta$10	6

END OF SEARCH HISTORY

STN SEARCH **09/931,186**

6/15/04

```
=> file .nash
=> s crystal? and cystine and mut?
L1          41 FILE MEDLINE
L2          39 FILE CAPLUS
L3          50 FILE SCISEARCH
L4          10 FILE LIFESCI
L5          24 FILE BIOSIS
L6          23 FILE EMBASE

TOTAL FOR ALL FILES
L7         187 CRYSTAL? AND CYSTINE AND MUT?

=> s l7 not 2002-2004/py
TOTAL FOR ALL FILES
L14        152 L7 NOT 2002-2004/PY

=> s l14 not knot
L16         24 FILE MEDLINE
L17         26 FILE CAPLUS
L18         24 FILE SCISEARCH
L19          7 FILE LIFESCI
L20         11 FILE BIOSIS
L21         12 FILE EMBASE

TOTAL FOR ALL FILES
L22        104 L14 NOT KNOT

=> dup rem l22
PROCESSING COMPLETED FOR L22
L23          58 DUP REM L22 (46 DUPLICATES REMOVED)

=> s l22 and x-ray
L24         10 FILE MEDLINE
L25          6 FILE CAPLUS
L26          4 FILE SCISEARCH
L27          2 FILE LIFESCI
L28          2 FILE BIOSIS
L29          3 FILE EMBASE

TOTAL FOR ALL FILES
L30         27 L22 AND X-RAY

=> dup rem l30
PROCESSING COMPLETED FOR L30
L31          14 DUP REM L30 (13 DUPLICATES REMOVED)

=> d 1-14 ibib abs

L31  ANSWER 1 OF 14     MEDLINE on STN                  DUPLICATE 1
ACCESSION NUMBER:   2001252653     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 11350172
TITLE:              What is the average conformation of bacteriophage T4
                    lysozyme in solution? A domain orientation study using
                    dipolar couplings measured by solution NMR.
AUTHOR:             Goto N K; Skrynnikov N R; Dahlquist F W; Kay L E
CORPORATE SOURCE:   Department of Biochemistry, University of Toronto, 1 King's
                    College Circle, Toronto, Ontario, M5S 1A8, Canada.
CONTRACT NUMBER:    GM57766 (NIGMS)
SOURCE:             Journal of molecular biology, (2001 May 11) 308 (4) 745-64.
                    Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY:       England: United Kingdom
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200106
ENTRY DATE:         Entered STN: 20010611
                    Last Updated on STN: 20010611
                    Entered Medline: 20010607
AB   Lysozyme from T4 bacteriophage is comprised of two domains that are both
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involved in binding substrate. Although wild-type lysozyme has been exclusively **crystallized** in a closed form that is similar to the peptidoglycan-bound conformation, a more open structure is thought to be required for ligand binding. To determine the relative arrangement of domains within T4 lysozyme in the solution state, dipolar couplings were measured in several different dilute liquid **crystalline** media by solution NMR methods. The dipolar coupling data were analyzed with a domain orientation procedure described previously that utilizes high-resolution **X-ray** structures. The cleft between the domains is significantly larger in the average solution structure than what is observed in the **X-ray** structure of the ligand-free form of the protein (approximately 17 degrees closure from solution to **X-ray** structures). A comparison of the solution domain orientation with **X-ray**-derived structures in the protein data base shows that the solution structure resembles a **crystal** structure obtained for the M6I **mutant**. Dipolar couplings were also measured on the lysozyme **mutant** T21C/T142C, which was oxidized to form an inter-domain disulfide bond (T4SS). In this case, the inter-domain solution structure was found to be more closed than was observed in the **crystal** (approximately 11 degrees). Direct refinement of lysozyme **crystal** structures with the measured dipolar couplings using the program CNS, establishes that this degree of closure can be accommodated whilst maintaining the inter-domain **cystine** bond. The differences between the average solution conformations obtained using dipolar couplings and the **crystal** conformations for both forms of lysozyme investigated in this study illustrate the impact that **crystal** packing interactions can have on the arrangement of domains within proteins and the importance of alternative methods to **X-ray crystallography** for evaluating inter-domain structure.

L31 ANSWER 2 OF 14 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 1999:328274 SCISEARCH
THE GENUINE ARTICLE: 188PR
TITLE: Chloroplast NADP-malate dehydrogenase: structural basis of light-dependent regulation of activity by thiol oxidation and reduction
AUTHOR: Carr P D (Reprint); Verger D; Ashton A R; Ollis D L
CORPORATE SOURCE: AUSTRALIAN NATL UNIV, RES SCH CHEM, POB 414, CANBERRA, ACT 2601, AUSTRALIA (Reprint); CSIRO, CANBERRA, ACT 2601, AUSTRALIA
COUNTRY OF AUTHOR: AUSTRALIA
SOURCE: STRUCTURE WITH FOLDING & DESIGN, (15 APR 1999) Vol. 7, No. 4, pp. 461-475.
Publisher: CURRENT BIOLOGY LTD, 34-42 CLEVELAND STREET, LONDON W1P 6LE, ENGLAND.
ISSN: 0969-2126.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 63

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: NADP-dependent malate dehydrogenase (EC 1.1.1.82) is a light-activated chloroplast enzyme that functions in the C-4 pathway of photosynthesis. The light regulation is believed to be mediated in vivo by thioredoxin-catalyzed reduction and re-oxidation of **cystine** residues. TI The rates of reversible activation and inactivation of the enzyme are strongly influenced by the coenzyme substrates that seem to ultimately determine the steady-state extent of activation in vivo.

Results: The **X-ray** structure of the inactive, oxidized enzyme was determined at 2.8 Angstrom resolution. The core structure is homologous to NAD-dependent malate dehydrogenases. Two surface-exposed and thioredoxin-accessible disulfide bonds are present, one in the N-terminal extension and the other in the C-terminal extension. The C-terminal peptide of the inactive, oxidized enzyme is constrained by its disulfide bond to fold into the active site over NADP(+), hydrogen bonding to the catalytic His225 as well as obstructing access of the C-4 acid substrate. Two loops flanking the active site, termed the Arg(2) and Trp loops, that contain the C-4 acid substrate binding residues are

prevented from closing by the C-terminal extension.
Conclusions: The structure explains the role of the C-terminal extension in inhibiting activity. The negative C terminus will interact more strongly with the positively charged nicotinamide of NADP(+) than NADPH, explaining why the coenzyme-binding affinities of the enzyme differ so markedly from those of all other homologous alpha-hydroxy acid dehydrogenases. NADP(+) may also slow dissociation of the C terminus upon reduction, providing a mechanism for the inhibition of activation by NADP(+) but not NADPH.

L31 ANSWER 3 OF 14 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1998128784 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9466900
TITLE: Voltage-gating of Escherichia coli porin: a **cystine** -scanning **mutagenesis** study of loop 3.
AUTHOR: Bainbridge G; Mobasheri H; Armstrong G A; Lea E J; Lakey J H
CORPORATE SOURCE: Department of Biochemistry and Genetics, Medical School, University of Newcastle Upon Tyne, UK.
SOURCE: Journal of molecular biology, (1998 Jan 16) 275 (2) 171-6. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980312
Last Updated on STN: 20000303
Entered Medline: 19980227
AB Porins, such as Escherichia coli OmpF, provide the only reported example of a voltage-gated channel where the three-dimensional structure is known to high resolution. **Mutations** that affect voltage-gating are clustered around the eyelet region, which is a mid-channel constriction caused by a polypeptide loop (L3) folding inside the lumen of this beta-barrel pore. These data, combined with molecular dynamics simulations, indicate that voltage-gating may involve L3 displacement. We have constructed six double cysteine OmpF **mutants**, five of which form disulphide bonds fixing L3 in the conformation determined by **X-ray crystallography**. These channels have altered single-channel conductances but unimpaired voltage-gating. The data show that L3 movement is not required for voltage-gating.

L31 ANSWER 4 OF 14 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 1998385862 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9720908
TITLE: Displacement of OmpF loop 3 is not required for the membrane translocation of colicins N and A in vivo.
AUTHOR: Bainbridge G; Armstrong G A; Dover L G; Whelan K F; Lakey J H
CORPORATE SOURCE: Department of Biochemistry and Genetics, The Medical School, The University of Newcastle upon Tyne, UK.
SOURCE: FEBS letters, (1998 Aug 7) 432 (3) 117-22. Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19981006
Last Updated on STN: 19981006
Entered Medline: 19980918
AB The pore-forming colicins N and A require the porin, OmpF, in order to translocate across the outer membrane of Escherichia coli. We investigated the hypothesis that in vivo, colicins N and A may traverse the outer membrane through the OmpF channel. In order to accommodate a polypeptide in the pore, the mid-channel constriction loop of OmpF, L3, would need to undergo a conformational change. We used five OmpF **cystine mutants**, which fix L3 in the conformation determined by **X-ray crystallography**, to investigate L3 movement during colicin activity in vivo. Sensitivity to colicins N and A of E. coli cells expressing these OmpF **cystine**

mutants was determined using cell survival and in vivo potassium efflux and fluorescence assays. Results indicate that gross movement of L3 is not required for colicin N or A activity and that neither of these colicins crosses the outer membrane of E. coli through the lumen of the OmpF pore.

L31 ANSWER 5 OF 14 MEDLINE on STN
ACCESSION NUMBER: 97345946 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9201965
TITLE: Structures of Cys319 variants and acetohydroxamate-inhibited Klebsiella aerogenes urease.
AUTHOR: Pearson M A; Michel L O; Hausinger R P; Karplus P A
CORPORATE SOURCE: Section of Biochemistry, Cornell University, Ithaca, New York 14853, USA.
SOURCE: Biochemistry, (1997 Jul 1) 36 (26) 8164-72.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1FWA; PDB-1FWB; PDB-1FWC; PDB-1FWD; PDB-1FWE; PDB-1FWF; PDB-1FWG; PDB-1FWH; PDB-3KAU
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970805
Last Updated on STN: 20000303
Entered Medline: 19970721

AB Cys319 is located on a mobile flap covering the active site of Klebsiella aerogenes urease but does not play an essential role in catalysis. Four urease variants altered at position C319 range from having high activity (C319A) to no measurable activity (C319Y), indicating Cys is not required at this position, but its presence is highly influential [Martin, P. R., & Hausinger, R. P. (1992) J. Biol. Chem. 267, 20024-20027]. Here, we present 2.0 A resolution **crystal** structures of C319A, C319S, C319D, and C319Y proteins and the C319A variant inhibited by acetohydroxamic acid. These structures show changes in the hydration of the active site nickel ions and in the position and flexibility of the active site flap. The C319Y protein exhibits an alternate conformation of the flap, explaining its lack of activity. The changes in hydration and conformation suggest that there are suboptimal protein-solvent and protein-protein interactions in the empty urease active site which contribute to urease catalysis. Specifically, we hypothesize that the suboptimal interactions may provide a significant source of substrate binding energy, and such hidden energy may be a common phenomenon for enzymes that contain mobile active site loops and undergo an induced fit. The acetohydroxamic acid-bound structure reveals a chelate interaction similar to those seen in other metalloenzymes and in a small molecule nickel complex. The inhibitor binding mode supports the proposed mode of urea binding. We complement these structural studies with extended functional studies of C319A urease to show that it has enhanced stability and resistance to inhibition by buffers containing nickel ions. The near wild-type activity and enhanced stability of the C319A variant make it useful for further studies of urease structure-function relationships.

L31 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:165596 CAPLUS
DOCUMENT NUMBER: 126:247933
TITLE: **Crystal** structures of modified Apo-His117Gly and Apo-His46Gly **mutants** of Pseudomonas aeruginosa azurin
AUTHOR(S): Hammann, Christian; van Pouderoyen, Gertie; Nar, Herbert; Rueth, Franz-Xaver Gomis; Messerschmidt, Albrecht; Huber, Robert; den Blaauwen, Tanneke; Canters, Gerard W.
CORPORATE SOURCE: Max Planck Inst. Biochemie, Munich, Germany
SOURCE: Journal of Molecular Biology (1997), 266(2), 357-366
CODEN: JMOBAK; ISSN: 0022-2836
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The **x-ray crystal** structures of two metal ligand **mutants** of azurin from Pseudomonas aeruginosa have been

solved. In both **mutants** (His117Gly and His46Gly azurin) one of the copper coordinating histidine residues is replaced by a glycine, creating an empty space in the coordination sphere of the copper ion. The **crystal** structure of His117Gly azurin at 2.4 .ANG. resoln. showed that this **mutant** had undergone partial oxidn. at the disulfide bridge between Cys3 and Cys26 and full oxidn. at the copper ligand Cys112. There is no copper present in the **crystd.** form and the bulky group of the oxidized cysteine at position 112 causes large structural rearrangements in the protein structure, esp. in the loops connecting the .beta.-sheets. In the structure of the wild-type holo-azurin from P. aeruginosa the hydrophobic patch is important for the packing of the azurin mols. into dimers which then arrange into tetramers. The completely different packing of the apo-His117Gly **mutant** can be explained by the disruption of the hydrophobic patch area by the **mutation**-induced main-chain conformational change of residues 112 to 115. The structure of apo-His46Gly azurin at 2.5 .ANG. resoln. is the same as the wild-type structure except for the immediate environment at the site of the **mutation**. In the His46Gly structure water mols. are found at positions that in the wild-type structure are occupied by the imidazole ring of His46 and the copper ion. The imidazole ring of His117 is shifted by about 1 .ANG. towards the surface of the protein, similar to that obsd. for 50% of the mols. in the wild-type apo-azurin structure. This shift causes a slight rearrangement of the monomers within the tetramer such that one local dyad becomes a **crystallog.** dyad parallel to the c-axis. This leads to a change in the space group from P212121 to P21,12.

L31 ANSWER 7 OF 14 MEDLINE on STN
ACCESSION NUMBER: 96230477 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8674705
TITLE: Structure and mechanism of GTP cyclohydrolase I of Escherichia coli.
AUTHOR: Nar H; Huber R; Meining W; Bracher A; Fischer M; Hosl C; Ritz H; Schmid C; Weinkauf S; Bacher A
CORPORATE SOURCE: Max-Planck-Institut fur Biochemie, Martinsried, Federal Republic of Germany.
SOURCE: Biochemical Society transactions, (1996 Feb) 24 (1) 37S. Journal code: 7506897. ISSN: 0300-5127.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960822
Last Updated on STN: 20000303
Entered Medline: 19960809

L31 ANSWER 8 OF 14 MEDLINE on STN
ACCESSION NUMBER: 96072753 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7578129
TITLE: Solution conformation of the extracellular domain of the human tumor necrosis factor receptor probed by Raman and UV-resonance Raman spectroscopy: structural effects of an engineered PEG linker.
AUTHOR: Tuma R; Russell M; Rosendahl M; Thomas G J Jr
CORPORATE SOURCE: Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri, Kansas City 64110-2499, USA.
CONTRACT NUMBER: GM50776 (NIGMS)
SOURCE: Biochemistry, (1995 Nov 21) 34 (46) 15150-6. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199512
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 20000303
Entered Medline: 19951226

AB The solution structure of the Escherichia coli-expressed extracellular domain, residues 12-172, of the human 55 kDa type I tumor necrosis factor

receptor (TNFR) has been probed by Raman (514.5 nm) and ultraviolet-resonance Raman (244 nm) excitations. The Raman spectra have been collected from both the free TNFR domain and an engineered "dumbbell-like" derivative, consisting of two **mutant** receptor moieties linked by a 20 kDa polyethylene glycol (PEG) tether. The results demonstrate a TNFR secondary structure which is rich in beta-sheet and deficient in alpha-helix, consistent with the reported **X-ray crystal** structure of baculovirus expressed receptor complexed with factor beta [Banner, D. W., D'Arcy, A., Janes, W., Gentz, R., Schoenfeld, H.-J., Broger, C., Loetscher, H., & Lesslauer, W. (1993) Cell 73, 431-445]. Conversely, the solution structure of TNFR differs from the **crystal** structure in its distribution of disulfide rotamers and in the orientation of its unique indole side chain (tryptophan-107). These differences are attributed, respectively, to N-terminal truncation and factor binding in the TNFR **crystal** structure. The tryptophan configuration, which is easily monitored in both Raman and UVRR spectra, is proposed as a potential signal of receptor/factor recognition and binding. Application of the Raman probes to the engineered TNFR dumbbell, which is of interest as a potential therapeutic, shows that TNFR moieties of the dumbbell exhibit secondary structures and side chain environments which are indistinguishable from those of the native, wild-type moiety. The results suggest that the PEGylated dumbbell may function as an effective TNFR drug delivery system without the consequence of a deleterious antigenic response.

L31 ANSWER 9 OF 14 MEDLINE on STN
ACCESSION NUMBER: 96109217 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8618856
TITLE: Active site topology and reaction mechanism of GTP cyclohydrolase I.
AUTHOR: Nar H; Huber R; Auerbach G; Fischer M; Hosl C; Ritz H; Bracher A; Meining W; Eberhardt S; Bacher A
CORPORATE SOURCE: Max-Planck-Institut fur Biochemie, Martinsried, Federal Republic of Germany.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1995 Dec 19) 92 (26) 12120-5. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960620
Last Updated on STN: 19970203
Entered Medline: 19960607

AB GTP cyclohydrolase I of Escherichia coli is a torus-shaped homodecamer with D5 symmetry and catalyzes a complex ring expansion reaction conducive to the formation of dihydroneopterin triphosphate from GTP. The **x-ray** structure of a complex of the enzyme with the substrate analog, dGTP, bound at the active site was determined at a resolution of 3 A. In the decamer, 10 equivalent active sites are present, each of which contains a 10-A deep pocket formed by surface areas of 3 adjacent subunits. The substrate forms a complex hydrogen bond network with the protein. Active site residues were modified by site-directed **mutagenesis**, and enzyme activities of the **mutant** proteins were measured. On this basis, a mechanism of the enzyme-catalyzed reaction is proposed. Cleavage of the imidazole ring is initiated by protonation of N7 by His-179 followed by the attack of water at C8 of the purine system. **Cystine** Cys-110 Cys-181 may be involved in this reaction step. Opening of the imidazole ring may be in concert with cleavage of the furanose ring to generate a Schiff's base from the glycoside. The gamma-phosphate of GTP may be involved in the subsequent Amadori rearrangement of the carbohydrate side chain by activating the hydroxyl group of Ser-135.

L31 ANSWER 10 OF 14 MEDLINE on STN
ACCESSION NUMBER: 95218619 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7703837
TITLE: Nerve growth factor: structure/function relationships.
AUTHOR: Bradshaw R A; Murray-Rust J; Ibanez C F; McDonald N Q; Lapatto R; Blundell T L

CORPORATE SOURCE: Department of Biological Chemistry, College of Medicine, University of California, Irvine 92717.
SOURCE: Protein science : a publication of the Protein Society, (1994 Nov) 3 (11) 1901-13.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950518
Last Updated on STN: 19950518
Entered Medline: 19950509

AB Nerve growth factor (NGF), which has a tertiary structure based on a cluster of 3 **cystine** disulfides and 2 very extended, but distorted beta-hairpins, is the prototype of a larger family of neurotrophins. Prior to the availability of cloning techniques, the mouse submandibular gland was the richest source of NGF and provided sufficient material to enable its biochemical characterization. It binds as a dimer to at least 2 cell-surface receptor types expressed in a variety of neuronal and non-neuronal cells. Residues involved in these interactions and in the maintenance of tertiary and quaternary structure have been identified by chemical modification and site-directed **mutagenesis** , and this information can be related to their location in the 3-dimensional structure. For example, interactions between aromatic residues contribute to the stability of the NGF dimer, and specific surface lysine residues participate in receptor contacts. The conclusion from these studies is that receptor interactions involve broad surface regions, which may be composed of residues from both promoters in the dimer.

L31 ANSWER 11 OF 14 MEDLINE on STN
ACCESSION NUMBER: 94002000 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8399159
TITLE: Structure of an engineered His3Cys zinc binding site in human carbonic anhydrase II.
AUTHOR: Ippolito J A; Christianson D W
CORPORATE SOURCE: Department of Chemistry, University of Pennsylvania, Philadelphia 19104-6323.
CONTRACT NUMBER: GM08275 (NIGMS)
SOURCE: Biochemistry, (1993 Sep 28) 32 (38) 9901-5.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199311
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 20000303
Entered Medline: 19931102

AB **X-ray crystallographic** analysis of the Thr-199-->Cys (T199C) variant of human carbonic anhydrase II reveals the first high-resolution structure of an engineered zinc coordination polyhedron in a metalloenzyme. In the wild-type enzyme, Thr-199 accepts a hydrogen bond from zinc-bound hydroxide; in the variant, the polypeptide backbone is sufficiently plastic to permit Cys-199 to displace hydroxide ion and coordinate to zinc with nearly perfect coordination stereochemistry. Importantly, the resulting His3-Cys-Zn2+ motif binds zinc more tightly than the wild-type enzyme [Kiefer, L. L., Krebs, J. F., Paterno, S. A., & Fierke C. A. (1993) Biochemistry (preceding paper in this issue)]. This novel zinc coordination polyhedron is analogous to that postulated for matrix metalloproteinase zymogens such as prostromelysin, where a cysteine-zinc interaction is responsible for the inactivity of the zymogen. Intriguingly, Cys-199 of T199C CAII is displaced from zinc coordination by soaking **crystals** in high concentrations of acetazolamide. Hence, residual catalytic activity measured for this variant probably arises from an alternate conformer of Cys-199 which allows the catalytic nucleophile, hydroxide ion, to be activated by zinc coordination.

L31 ANSWER 12 OF 14 MEDLINE on STN

ACCESSION NUMBER: 88162883 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3126743
TITLE: Site-directed **mutagenesis** of the alpha subunit of tryptophan synthase from Salmonella typhimurium.
AUTHOR: Ahmed S A; Kawasaki H; Bauerle R; Morita H; Miles E W
CORPORATE SOURCE: Laboratory of Biochemical Pharmacology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland 20892.
SOURCE: Biochemical and biophysical research communications, (1988 Mar 15) 151 (2) 672-8.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198804
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 20000303
Entered Medline: 19880414

AB Site-specific **mutagenesis** has been used to prepare two **mutant** forms of the alpha subunit of tryptophan synthase from Salmonella typhimurium in which either cysteine-81 or cysteine-118 is replaced by a serine residue. These **mutant** proteins are potentially useful for **x-ray crystallographic** studies since a heavy metal binding site is specifically eliminated in each **mutant**. The purified **mutant** proteins are fully active in four reactions catalyzed by the wild type alpha 2 beta 2 complex of tryptophan synthase. However, the **mutant** alpha 2 beta 2 complexes dissociate more readily and are less heat-stable than the wild type alpha 2 beta 2 complex. Thus, cysteine-81 and cysteine-118 of the alpha subunit serve structural but not functional roles.

L31 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1970:195 CAPLUS
DOCUMENT NUMBER: 72:195
TITLE: PtCl2-4: a methionine-specific label for protein **crystallography**
AUTHOR(S): Dickerson, Richard E.; Eisenberg, D.; Varnum, J. C.; Kopka, M. L.
CORPORATE SOURCE: California Inst. of Technol., Pasadena, CA, USA
SOURCE: Journal of Molecular Biology (1969), 45(1), 77-84
CODEN: JMOBAK; ISSN: 0022-2836
DOCUMENT TYPE: Journal
LANGUAGE: English

AB PtCl42- has been found in six **cryst.** proteins to bind selectively to methionine side groups. **X-ray** evidence in horse heart ferricytochrome c suggests that the square-planar Pt(II) is oxidized to octahedral Pt(IV) with the methionine S acting as a fifth ligand by donating either one or the other of its lone electron pairs in two **mutually** exclusive Pt binding positions. Evolutionary criteria are proposed for choosing proteins with a high probability of specific heavy-atom labeling at methionine, **cystine**, and histidine.

L31 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1948:8902 CAPLUS
DOCUMENT NUMBER: 42:8902
ORIGINAL REFERENCE NO.: 42:1984b-h
TITLE: Methionine synthesis in Neurospora. Isolation of cystathionine
AUTHOR(S): Horowitz, N. H.
CORPORATE SOURCE: California Inst. Technol., Pasadena
SOURCE: Journal of Biological Chemistry (1947), 171, 255-64
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
GI For diagram(s), see printed CA Issue.

AB Four **x-ray** induced **mutant** strains of N. crassa are unable to synthesize methionine (I) and require it for growth. Each of the **mutants** (me-1, me-2, me-3, and me-4) are different single-gene **mutants** unable to carry out some single step in the

synthesis of I. The following steps were found to occur in the biosynthesis of I and were blocked at that step in the appropriate **mutant**: gene me-4 .fwdarw. cysteine gene me-3 .fwdarw. intermediate gene me-2 .fwdarw. homocysteine gene me-1 .fwdarw. methionine. Genes me-2 and me-3 are nonallelic, as evidenced by the fact that the me-2 block allows accumulation of a precursor or intermediate of homocysteine which can be utilized by strain me-3, but not by me-2. This precursor was isolated and identified as cystathionine as follows: Strain me-2 was grown for 7-10 days at 25.degree. under forced aeration in 16 l. basal medium containing 0.4 g. of DL-methionine. The washed mycelium (290-360 g. moist wt.) contained 10 times more precursor than the medium. The mycelium was dispersed in H2O and poured into 10 l. boiling H2O per kg. of moist mold, filtered through cloth, and the extn. repeated. The combined filtrates adjusted to pH 5 (dil. HCl), were placed in the cold overnight and the ppt. filtered and discarded. After neutralization the filtrate was concd. under reduced pressure to 2.6 l. per kg. of original mycelium. One vol. of 95% alc. was added, and the ppt. which developed overnight in the cold was discarded. The filtrate was concd. to 550 ml. per kg. original mycelium and the ppt. resulting from addn. of 5 vols. alc. was collected by centrifugation, washed, dissolved in hot H2O, concd. under reduced press. to incipient **crystn**., 3 vol. alc. added, and **crystn**. allowed to occur overnight in the cold. Yield, 360 mg. colorless octagonal prisms per kg. moist mycelium. The yield was doubled in one batch by supplementing the basal medium with 0.2 g. L-**cystine** and 0.2 g. DL-homoserine in addn. to methionine. This purified substance darkens at 270.degree. and m. 301.degree., [.alpha.]D25 of a 1% soln. in 1 N HCl is 26 .+-. 2.degree.. These properties, plus elementary analysis and dibenzoyl deriv., m. 228-9.degree., are in agreement with those obtained for synthetic L-cystathionine. The purified compd. has the same activity as synthetic cystathionine in supporting growth of strain me-3, in absence of I. The mol. activity of cystathionine for strain me-3 is approx. 0.6 that of I. L- and D-Allocystathionine and D-cystathionine are inactive.

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=> s crystal? and selenocystine
L32           2 FILE MEDLINE
L33           7 FILE CAPLUS
L34           1 FILE SCISEARCH
L35           0 FILE LIFESCI
L36           1 FILE BIOSIS
L37           1 FILE EMBASE

TOTAL FOR ALL FILES
L38          12 CRYSTAL? AND SELENOCYSTINE

=> s l38 not 2002-2004/py
L39           2 FILE MEDLINE
L40           7 FILE CAPLUS
L41           1 FILE SCISEARCH
L42           0 FILE LIFESCI
L43           1 FILE BIOSIS
L44           1 FILE EMBASE

TOTAL FOR ALL FILES
L45          12 L38 NOT 2002-2004/PY

=> dup rem l45
PROCESSING COMPLETED FOR L45
L46           8 DUP REM L45 (4 DUPLICATES REMOVED)

=> d l46 1-8 ibib abs

L46  ANSWER 1 OF 8     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:    2001353268     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 11305255
TITLE:               Allium chemistry: synthesis, natural occurrence, biological
                     activity, and chemistry of Se-alk(en)ylselenocysteines and
                     their gamma-glutamyl derivatives and oxidation products.
AUTHOR:              Block E; Birringer M; Jiang W; Nakahodo T; Thompson H J;
                     Toscano P J; Uzar H; Zhang X; Zhu Z
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CORPORATE SOURCE: Department of Chemistry, State University of New York, Albany 12222, USA.. eb801@csc.albany.edu
CONTRACT NUMBER: CA45164 (NCI)
SOURCE: Journal of agricultural and food chemistry, (2001 Jan) 49 (1) 458-70.
Journal code: 0374755. ISSN: 0021-8561.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010625
Last Updated on STN: 20010625
Entered Medline: 20010621

AB Syntheses are reported for gamma-glutamyl Se-methylselenocysteine (Sa), selenolanthionine (16), Se-1-propenylselenocysteine (Gd), Se-2-methyl-2-propenyl-L-selenocysteine (6e), and Se-2-propynyl-L-selenocysteine (6f). Oxidation of 8a and Se-methylselenocysteine (Ga) gives methaneseleninic acid (24), characterized by X-ray **crystallography**, and dimethyl diselenide (25). Oxidation of Se-2-propenyl-L-selenocysteine (6c) gives allyl alcohol and 3-seleninoalanine (22). Compound 22 is also formed on oxidation of 16 and **selenocystine** (4). Oxidation of 6d gives 2-[(E,Z)-1-propenylseleno]propanal (36). These oxidations occur by way of selenoxides, detected by chromatographic and spectroscopic methods. The natural occurrence of many of the Se-alk(en)ylselenocysteines and their gamma-glutamyl derivatives and oxidation products is discussed. Three homologues of the potent cancer chemoprevention agents 6a and 6c, namely 6d-f, were evaluated for effects on cell growth, induction of apoptosis, and DNA-damaging activity using two murine mammary epithelial cell lines. Although each compound displays a unique profile of activity, none of these compounds (Gd-f) is likely to exceed the chemopreventive efficacy of selenocysteine Se-conjugates Ga and 6c.

L46 ANSWER 2 OF 8 MEDLINE on STN
ACCESSION NUMBER: 90228368 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2184035
TITLE: Selenomethionyl proteins produced for analysis by multiwavelength anomalous diffraction (MAD): a vehicle for direct determination of three-dimensional structure.
AUTHOR: Hendrickson W A; Horton J R; LeMaster D M
CORPORATE SOURCE: Howard Hughes Medical Institute, Columbia University, New York, NY 10032.
CONTRACT NUMBER: GM34102 (NIGMS)
SOURCE: EMBO journal, (1990 May) 9 (5) 1665-72.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199005
ENTRY DATE: Entered STN: 19900706
Last Updated on STN: 20000303
Entered Medline: 19900531

AB An expression system has been established for the incorporation of selenomethionine into recombinant proteins produced from plasmids in Escherichia coli. Replacement of methionine by selenomethionine is demonstrated at the level of 100% for both T4 and E. coli thioredoxins. The natural recombinant proteins and the selenomethionyl variants of both thioredoxins **crystallize** isomorphously. Anomalous scattering factors were deduced from synchrotron X-ray absorption measurements of **crystals** of the selenomethionyl proteins. Taken with reference to experience in the structural analysis of selenobiotinyl streptavidin by the method of multiwavelength anomalous diffraction (MAD), these data indicate that recombinant selenomethionyl proteins analyzed by MAD phasing offer a rather general means for the elucidation of atomic structures.

L46 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 1975:96725 CAPLUS
DOCUMENT NUMBER: 82:96725
TITLE: Efficacy of selenium in selenium compounds and feeds

for prevention of pancreatic fibrosis in chicks
AUTHOR(S): Cantor, Austin H.; Langevin, Marilyn L.; Noguchi, Tadashi; Scott, Milton L.
CORPORATE SOURCE: Dep. Poult. Sci., Cornell Univ., Ithaca, NY, USA
SOURCE: Journal of Nutrition (1975), 105(1), 106-11
CODEN: JONUAI; ISSN: 0022-3166
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The efficacy of dietary supplements of Se provided by Na selenite, selenomethionine, **selenocystine**, wheat, and tuna meal was evaluated for prevention of pancreatic fibrosis in chicks fed a **crystalline** amino acid basal diet contg. 0.012 ppm of naturally occurring Se and 15 IU vitamin E/kg diet. Histol. examn. of the pancreases indicated that wheat and selenomethionine were the most effective sources of Se. The Se compds. were reevaluated in a subsequent expt. using a basal diet supplemented with 100 IU vitamin E/kg of diet. Selenomethionine was 4 times as effective as either selenite or **selenocystine** with respect to prevention of pancreatic degeneration and increasing the relative wt. and Se concn. of the pancreas. Studies on plasma and pancreatic glutathione peroxidase activities did not show any relationship between enzyme activity and prevention of the deficiency disease.

L46 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1970:51211 CAPLUS
DOCUMENT NUMBER: 72:51211
TITLE: Properties of **crystalline** serine dehydratase of rat liver
AUTHOR(S): Nakagawa, Hachiro; Kimura, Hiroshi
CORPORATE SOURCE: Inst. Protein Res., Osaka Univ., Osaka, Japan
SOURCE: Journal of Biochemistry (Tokyo, Japan) (1969), 66(5), 669-83
CODEN: JOBIAO; ISSN: 0021-924X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Serine dehydratase [EC 4.2.1.13] was **crystallized** from rat liver. **Cryst.** enzyme was homogeneous and had an s20,w of 4.25. Its mol. wt. was calcd. to be 64,000 by the sedimentation equil. method. In the ouchterlony gel double diffusion test, **cryst.** enzyme behaved as a s ingle band against the antiserine dehydratase serum obtained from rabbits immunized with the same prepn. **Crystd.** enzyme moved as a single component on pH gradient electrophoresis and its isoelectric point was detd. to be pH 6.7. However, the prepn. was sepd. into 2 fractions by starch zone electrophoresis. Serine dehydratase was shown to be the same enzyme as threonine dehydratase but it differed from cystathionine dehydratase. The Km values for L-serine and L-threonine were detd. to be 5.7 .times. 10-2M and 8.7 .times. 10-2M, resp. The enzyme requires pyridoxal phosphate and a sulfhydryl compd. The dissocn. const. of pyridoxal phosphate was 3.7 .times. 10-7M. **Crystd.** enzyme contained 2 moles of pyridoxal phosphate per mole of enzyme. Serine dehydratase was strongly inhibited by DL-**selenocystine**.

L46 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1970:529110 CAPLUS
DOCUMENT NUMBER: 73:129110
TITLE: Toxicology and metabolic fate of selenium in sheep
AUTHOR(S): Neethling, L. P.; Brown, John M. M.; De Wet, P. J.
CORPORATE SOURCE: Vet. Res. Inst., Onderstepoort, S. Afr.
SOURCE: Journal of the South African Veterinary Medical Association (1968), 39(3), 25-33
CODEN: JSAVAI; ISSN: 0038-2809
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Fatal and (or) chronic doses of SeO2, selenomethionine (I), and **selenocystine** and their metabolic fate in Merino sheep and lambs were detd.; intoxication symptoms are described; the postulate that Se intoxication plays a role in the etiol. of the ovine hemolytic syndromes geeldikkop and enzootic icterus was confirmed by the appearance of the abnormal Hb C in circulating erythrocytes. Se urinary and biliary excretion were studied. Taurine-75Se was formed in vivo from I-75Se. Se was incorporated into plasma and tissue globulins, connective tissue,

mitochondria, aldolase, phosphorylase, and alc. dehydrogenase. **Cryst**. ovine Hb-75Se was isolated.

L46 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1967:473863 CAPLUS
DOCUMENT NUMBER: 67:73863
TITLE: Syntheses and pharmacological properties of selenium isologs of oxytocin and deaminooxytocin
AUTHOR(S): Walter, Roderich; Chan, Wah-Yip
CORPORATE SOURCE: Cornell Univ. Med. Coll., New York, NY, USA
SOURCE: Journal of the American Chemical Society (1967), 89(15), 3892-8
CODEN: JACSAT; ISSN: 0002-7863
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Isologs, differing from oxytocin (I) and deaminooxytocin (Ia) only in the replacement of the S in position 1 or 6, were synthesized, purified by countercurrent distribution, and tested for pharmacol. activities. For the synthesis of the isolog 1-hemi-L-**selenocystine**-oxytocin (II), N-carbobenzoxy-Se-benzyl-L-selenocysteine (III) was employed. This intermediate was obtained by the resolution of N-acetyl-Se-benzyl-DL-selenocysteine with hog acylase to yield Se-benzyl-L-selenocysteine which was subsequently carbobenzoxylated. The deamino-1-hemiselenooxytocin (IV) was synthesized with the use of Se-benzyl-.beta.-selenopropionic acid in place of III. In addn., 6-hemi-L-selenocystineoxytocin (V) and its deamino analog (VI) were prepd. For the synthesis of the intermediate tetrapeptide N-carbobenzoxy-Se-benzyl-L-selenocysteinyl-L-proly-L-leucylglycinamide, the tripeptide L-prolyl-L-leucylglycinamide was acylated with p-nitrophenyl N-carbobenzoxy-Se-benzyl-DL-selenocysteinate, and the resulting diastereoisomeric tetrapeptides were resolved by fractional **crystn**. Replacement of 1 S atom by a Se atom in postion 1 or 6 yielded highly potent isologs of I and Ia. Upon bioassay, II was found to possess 362 .+-. 9 units/mg. rat oxytocic activity, 351 .+-. 15 units/mg. rabbit milk-ejecting activity, 361 .+-. 18 units/mg. avian vasodepressor activity, 3.1 .+-. 0.2 units/mg. rat pressor activity, and 5.7 .+-. 0.6 units/mg. rat antidiuretic activity. IV exhibited 560 .+-. 34 units/mg. oxytocic activity, 248 .+-. 8 units/mg. milk-ejecting activity, 613 .+-. 38 units/mg. vasodepressor ac tivity, 1.7 .+-. 0.2 units/mg. pressor activity, and 24.0 .+-. 1.5 units/mg. antidiuretic activity. The comparable values for V and VI were 405 .+-. 5, 398 .+-. 6, 385 .+-. 15, 3.8 .+-. 0.2, and 3.4 .+-. 0.1 units/mg. and 492 .+-. 5, 397 .+-. 8, 622 .+-. 18, 1.1 .+-. 0.1, and 15.5 .+-. 1.0 units/mg., resp. 41 references.

L46 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1962:53674 CAPLUS
DOCUMENT NUMBER: 56:53674
ORIGINAL REFERENCE NO.: 56:10263d-h
TITLE: Convenient synthesis of inactive **selenocystine**
AUTHOR(S): Zdansky, Goran
CORPORATE SOURCE: Univ. Uppsala, Swed.
SOURCE: Arkiv. Kemi (1961), 17, 273-9
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB Inactive **selenocystine** (I) was prepd. by treating PhCH2SeH (II) with Me .alpha.-(acetylamino)acrylate (III) to give PhCH2SeCH2(AcNH)CHCO2Me (IV) which was saponified, reduced with Na in liquid NH3, oxidized, and deacetylated to yield I. Freshly distd. II (34.2 g.) and 28.6 g. III were mixed in the presence of a small amt. NaOMe and maintained at 100.degree. 10 hrs. Recrystn. of the **cryst**. residue left on cooling gave 94.7% IV, m. 88.5-90.degree.. The S analog was prepd. in similar fashion from III and PhCH2SH in 69% yield, m. 84-4.8.degree. (Hellmann and Folz, CA 52, 1927b). IV (4.29 g.) was dissolved in 16.7 cc. N NaOH at 40-50.degree., 5 cc. H2O added, and the mixt. left at room temp. 12 hrs. The turbid soln. was extd. twice with Et2O (10 cc.) and the pH of the aq. phase brought to 5.5 with dil. HCl. The ppt. was filtered after a few hrs. cooling, washed with ice H2O, dried, and recrystd. from 38% aq. EtOH to give 3.55 g. PhCH2SeCH2(AcNH)CHCO2H (V), m. 152-3.degree.. The S analog was prepd. in 93.7% yield. V (1.49 g.) was suspended in 40 cc. liquid NH3 and Na pieces added with stirring till a blue color persisted 10 min. Dry NH4Cl (0.4

g.) was added to decolorize the soln., the NH3 evapd., the residue dissolved in 25 cc. H2O, the pH adjusted to 7-8 with HCl, and the mixt. oxidized by adding 2 drops FeCl3 soln., and sucking air through with stirring. The black soln. turned brownish yellow after 40 min. Air was sucked in for another 10 min., the soln. extd. with 10 cc. Et2O, filtered, and refluxed with 6 cc. concd. HCl 2 hrs. The mixt. was evapd. in vacuo on a H2O bath, the residue dissolved in 50 cc. 2N HCl, and evapd. in vacuo to dryness. After repeating this once more, the residue was dissolved in 12 cc. H2O, the pH adjusted to 5.5 with 2N NaOH, filtered after 12 hrs. cooling, and the ppt. washed with H2O and EtOH and dried to give 87% I. Inactive cystine was prepd. similarly from the S analog of V. V and its S analog were also prepd. directly from the K salt of Me acetamido malonate and PhCH2SeH (or PhCH2SH), without isolation of the intermediates, in 51.5% yield.

L46 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1958:2292 CAPLUS
DOCUMENT NUMBER: 52:2292
ORIGINAL REFERENCE NO.: 52:479g-i,480a-b
TITLE: Analogs of acetyl chymotrypsin
AUTHOR(S): McDonald, C. E.; Balls, A. K.
CORPORATE SOURCE: Purdue Univ., West Lafayette, IN
SOURCE: Journal of Biological Chemistry (1957), 227, 727-36
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. C.A. 50, 15658g. Chymotrypsin was successfully acylated by the following nitrophenyl esters, m.p. of the esters given: 2,4-dinitrobenzoate, 130-1.degree.; (the remainder are p-nitrophenyl esters) hippurate, 170-1.degree.; isobutyrate, 39-40.degree.; trimethylacetate, 94-5.degree.; hydrocinnamate, 97-8.degree.. Both the speed of acylation and the stability of the product varied with the nature of the acyl group; both reactions were pH-dependent. When the acylating group contained a peptide linkage (hippuryl chymotrypsin), the acylated protein was decidedly less stable than Ac chymotrypsin; but when the acyl group was an aliphatic acid with a branched C chain, the stability was greatly increased in weakly alk. soln., e.g., trimethylacetyl chymotrypsin. Compared with other acyl chymotrypsins tested, the substance formed slowly and decompd. slowly. It is an excellent material for kinetic studies. It can be **crystd.**, reacts incompletely with HONH2 even in strongly alk. solns., and reverts slowly to chymotrypsin in the presence of tyrosine Et ester. It is readily reactivated at pH 8 under special conditions; both the milk-clotting and esterolytic properties of the original enzyme are recovered almost completely. The existence of a great variety of acylated chymotrypsins, whose individual stability varies between wide limits, appears possible. Thus, the very stable phosphorylated chymotrypsins, are at one end of such a scale while substituent groups contg. a peptide linkage are near the other.

=> s amyloid (3W) peptide (1w) binding protein or ERAB or hydroxyacyl-co dehydrogenase or hada2
L47 27 FILE MEDLINE
L48 49 FILE CAPLUS
L49 32 FILE SCISEARCH
L50 17 FILE LIFESCI
L51 39 FILE BIOSIS
L52 24 FILE EMBASE

TOTAL FOR ALL FILES
L53 188 AMYLOID (3W) PEPTIDE (1W) BINDING PROTEIN OR ERAB OR HYDROXYACYL -CO DEHYDROGENASE OR HADA2

=> s l53 and cysteine
L54 0 FILE MEDLINE
L55 1 FILE CAPLUS
L56 0 FILE SCISEARCH
L57 0 FILE LIFESCI
L58 0 FILE BIOSIS
L59 0 FILE EMBASE

TOTAL FOR ALL FILES
L60 1 L53 AND CYSTEINE

=> d ibib abs

L60 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:775265 CAPLUS
DOCUMENT NUMBER: 136:132090
TITLE: Investigation of differentially expressed genes during the development of mouse cerebellum
AUTHOR(S): Kagami, Yoshihiro; Furuichi, Teiichi
CORPORATE SOURCE: Laboratory for Molecular Neurogenesis, Brain Science Institute, RIKEN, Wako, 351-0198, Japan
SOURCE: Gene Expression Patterns (2001), 1(1), 39-59
CODEN: GEPEAD; ISSN: 1567-133X
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Before the discovery of DNA microarray and DNA chip technol., the expression of only a small no. of genes could be analyzed at a time. Currently, such technol. allows us the simultaneous anal. of a large no. of genes to systematically monitor their expression patterns that may be assocd. with various biol. phenomena. We utilized the Affymetrix GeneChip Mu11K to analyze the gene expression profile in developing mouse cerebellum to assist in the understanding of the genetic basis of cerebellar development in mice. Our anal. showed 81.6% (10.321/12.654) of the genes represented on the GeneChip were expressed in the postnatal cerebellum, and among those, 8.7% (897/10.321) were differentially expressed with more than a two-fold change in their max. and min. expression levels during the developmental time course. Further anal. of the differentially expressed genes that were clustered in terms of their expression patterns and the function of their encoded products revealed an aspect of the genetic foundation that lies beneath the cellular events and neural network formation that takes place during the development of the mouse cerebellum.
REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s l53 and muta?
L61 9 FILE MEDLINE
L62 14 FILE CAPLUS
L63 11 FILE SCISEARCH
L64 7 FILE LIFESCI
L65 12 FILE BIOSIS
L66 9 FILE EMBASE

TOTAL FOR ALL FILES
L67 62 L53 AND MUTA?

=> s l67 not 2002-2004/py
L68 7 FILE MEDLINE
L69 11 FILE CAPLUS
L70 9 FILE SCISEARCH
L71 6 FILE LIFESCI
L72 10 FILE BIOSIS
L73 7 FILE EMBASE

TOTAL FOR ALL FILES
L74 50 L67 NOT 2002-2004/PY

=> dup rem l74
PROCESSING COMPLETED FOR L74
L75 17 DUP REM L74 (33 DUPLICATES REMOVED)

=> d ibib abs

L75 ANSWER 1 OF 17 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2001296614 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11278849
TITLE: beta -Amyloid peptide-induced apoptosis regulated by a

novel protein containing a g protein activation module.
AUTHOR: Kajkowski E M; Lo C F; Ning X; Walker S; Sofia H J; Wang W; Edris W; Chanda P; Wagner E; Vile S; Ryan K; McHendry-Rinde B; Smith S C; Wood A; Rhodes K J; Kennedy J D; Bard J; Jacobsen J S; Ozenberger B A
CORPORATE SOURCE: Wyeth Neuroscience, Wyeth-Ayerst Research, CN 8000, Princeton, New Jersey 08543-8000, USA.
SOURCE: Journal of biological chemistry, (2001 Jun 1) 276 (22) 18748-56.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF353990; GENBANK-AF353991; GENBANK-AF353992; GENBANK-AF353993
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010730
Last Updated on STN: 20030105
Entered Medline: 20010726

AB Degeneration of neurons in Alzheimer's disease is mediated by beta-amyloid peptide by diverse mechanisms, which include a putative apoptotic component stimulated by unidentified signaling events. This report describes a novel beta-**amyloid peptide-binding protein** (denoted BBP) containing a G protein-coupling module. BBP is one member of a family of three proteins containing this conserved structure. The BBP subtype bound human beta-amyloid peptide in vitro with high affinity and specificity. Expression of BBP in cell culture induced caspase-dependent vulnerability to beta-amyloid peptide toxicity. Expression of a signaling-deficient dominant negative BBP **mutant** suppressed sensitivity of human Ntera-2 neurons to beta-amyloid peptide mediated toxicity. These findings suggest that BBP is a target of neurotoxic beta-amyloid peptide and provide new insight into the molecular pathophysiology of Alzheimer's disease.

=> d ibib abs 2-
YOU HAVE REQUESTED DATA FROM 16 ANSWERS - CONTINUE? Y/(N):y

L75 ANSWER 2 OF 17 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:897468 CAPLUS
DOCUMENT NUMBER: 136:130462
TITLE: hADA3 is required for p53 activity
AUTHOR(S): Wang, Ting; Kobayashi, Takahiko; Takimoto, Rishu; Denes, Alec E.; Snyder, Eric L.; El-Deiry, Wafik S.; Brachmann, Rainer K.
CORPORATE SOURCE: Division of Oncology, Department of Medicine, Washington University School of Medicine, St Louis, MO, 63110, USA
SOURCE: EMBO Journal (2001), 20(22), 6404-6413
CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The tumor suppressor protein p53 is a transcription factor that is frequently **mutated** in human cancers. In response to DNA damage, p53 protein is stabilized and activated by post-translational modifications that enable it to induce either apoptosis or cell cycle arrest. Using a novel yeast p53 dissociator assay, we identify hADA3, a part of histone acetyltransferase complexes, as an important cofactor for p53 activity. P53 and hADA3 phys. interact in human cells. This interaction is enhanced dramatically after DNA damage due to phosphorylation event(s) in the p53 N-terminus. Proper hADA3 function is essential for full transcriptional activity of p53 and p53-mediated apoptosis.

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L75 ANSWER 3 OF 17 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:158963 CAPLUS
DOCUMENT NUMBER: 135:4058

TITLE: Analysis of polymorphisms of AACT, **ERAB** and NACP genes in 15 patients with Alzheimer's disease
AUTHOR(S): Cui, Sisong; Yu, Zhengyan; Wu, Heng; Zhu, Hanmin; Meng, Guangxun; Hu, Gengxi
CORPORATE SOURCE: Shanghai Huadong Hospital/Shanghai Geriatrics Institute, Shanghai, 200040, Peop. Rep. China
SOURCE: Zhonghua Yixue Zazhi (Beijing, China) (2001), 81(2), 90-92
CODEN: CHHTAT; ISSN: 0376-2491
PUBLISHER: Zhonghua Yixue Zazhishe
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB The polymorphisms of the AACT, **ERAB** and NACP genes in Han Chinese Alzheimer's disease (AD) patients were analyzed. Denaturant gradient gel electrophoresis (DGGE) was used to scan all DNA fragments of 3 genes mentioned above in AD group (15 cases) and elderly control group (241 cases). A25G and G39A substitutions were found in the AACT gene. The gene frequency of A25G was 80% in the AD group and 47% in the control group ($P < 0.05$). The gene frequency of G39A genotype was 13% in the AD group and 0.4% in the control group ($P < 0.01$). The allele (39) frequencies were also significantly different between the 2 groups ($P < 0.01$). Any points with polymorphism in the **ERAB** and NACP genes were not found. A25G and G39A substitutions in the AACT gene were probably one of the risk factors to AD in Han Chinese.

L75 ANSWER 4 OF 17 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:775265 CAPLUS
DOCUMENT NUMBER: 136:132090
TITLE: Investigation of differentially expressed genes during the development of mouse cerebellum
AUTHOR(S): Kagami, Yoshihiro; Furuichi, Teiichi
CORPORATE SOURCE: Laboratory for Molecular Neurogenesis, Brain Science Institute, RIKEN, Wako, 351-0198, Japan
SOURCE: Gene Expression Patterns (2001), 1(1), 39-59
CODEN: GEPEAD; ISSN: 1567-133X
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Before the discovery of DNA microarray and DNA chip technol., the expression of only a small no. of genes could be analyzed at a time. Currently, such technol. allows us the simultaneous anal. of a large no. of genes to systematically monitor their expression patterns that may be assocd. with various biol. phenomena. We utilized the Affymetrix GeneChip Mu11K to analyze the gene expression profile in developing mouse cerebellum to assist in the understanding of the genetic basis of cerebellar development in mice. Our anal. showed 81.6% (10.321/12.654) of the genes represented on the GeneChip were expressed in the postnatal cerebellum, and among those, 8.7% (897/10.321) were differentially expressed with more than a two-fold change in their max. and min. expression levels during the developmental time course. Further anal. of the differentially expressed genes that were clustered in terms of their expression patterns and the function of their encoded products revealed an aspect of the genetic foundation that lies beneath the cellular events and neural network formation that takes place during the development of the mouse cerebellum.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L75 ANSWER 5 OF 17 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2000388554 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10809234
TITLE: GCN5 and ADA adaptor proteins regulate triiodothyronine/GRIP1 and SRC-1 coactivator-dependent gene activation by the human thyroid hormone receptor.
AUTHOR: Anafi M; Yang Y F; Barlev N A; Govindan M V; Berger S L; Butt T R; Walfish P G
CORPORATE SOURCE: Samuel Lunenfeld Research Institute, University of Toronto Medical School, Mount Sinai Hospital, Ontario, Canada.
SOURCE: Molecular endocrinology (Baltimore, Md.), (2000 May) 14 (5) 718-32.
Journal code: 8801431. ISSN: 0888-8809.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000818
Last Updated on STN: 20030215
Entered Medline: 20000810

AB We have used yeast genetics and in vitro protein-protein interaction experiments to explore the possibility that GCN5 (general control nonrepressed protein 5) and several other ADA (alteration/deficiency in activation) adaptor proteins of the multimeric SAGA complex can regulate T3/GRIP1 (glucocorticoid receptor interacting protein 1) and SRC-1 (steroid receptor coactivator-1) coactivator-dependent activation of transcription by the human T3 receptor beta1 (hTRbeta1). Here, we show that in vivo activation of a T3/GRIP1 or SRC-1 coactivator-dependent T3 hormone response element by hTRbeta1 is dependent upon the presence of yeast GCN5, ADA2, ADA1, or ADA3 adaptor proteins and that the histone acetyltransferase (HAT) domains and bromodomain (BrD) of yGCN5 must be intact for maximal activation of transcription. We also observed that hTRbeta1 can bind directly to yeast or human GCN5 as well as **hADA2**, and that the hGCN5(387-837) sequence could bind directly to either GRIP1 or SRC-1 coactivator. Importantly, the T3-dependent binding of hTRbeta1 to hGCN5(387-837) could be markedly increased by the presence of GRIP1 or SRC1. **Mutagenesis** of GRIP1 nuclear receptor (NR) Box II and III LXXLL motifs also substantially decreased both in vivo activation of transcription and in vitro T3-dependent binding of hTRbeta1 to hGCN5. Taken together, these experiments support a multistep model of transcriptional initiation wherein the binding of T3 to hTRbeta1 initiates the recruitment of p160 coactivators and GCN5 to form a trimeric transcriptional complex that activates target genes through interactions with ADA/SAGA adaptor proteins and nucleosomal histones.

L75 ANSWER 6 OF 17 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2000068830 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10600649
TITLE: Intrinsic alcohol dehydrogenase and hydroxysteroid dehydrogenase activities of human mitochondrial short-chain L-3-hydroxyacyl-CoA dehydrogenase.
AUTHOR: He X Y; Yang Y Z; Schulz H; Yang S Y
CORPORATE SOURCE: Department of Pharmacology, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314, USA.
CONTRACT NUMBER: AG04220 (NIA)
DK47392 (NIDDK)
HL30847 (NHLBI)
SOURCE: Biochemical journal, (2000 Jan 1) 345 Pt 1 139-43.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000229
Last Updated on STN: 20021210
Entered Medline: 20000214

AB The alcohol dehydrogenase (ADH) activity of human short-chain l-3-hydroxyacyl-CoA dehydrogenase (SCHAD) has been characterized kinetically. The k(cat) of the purified enzyme was estimated to be 2. 2 min(-1), with apparent K(m) values of 280 mM and 22microM for 2-propanol and NAD(+), respectively. The k(cat) of the ADH activity was three orders of magnitude less than the l-3-hydroxyacyl-CoA dehydrogenase activity but was comparable with that of the enzyme's hydroxysteroid dehydrogenase (HSD) activity for oxidizing 17beta-oestradiol [He, Merz, Mehta, Schulz and Yang (1999) J. Biol. Chem. 274, 15014-15019]. However, the k(cat) values of intrinsic ADH and HSD activities of human SCHAD were found to be two orders of magnitude less than those reported for endoplasmic-reticulum-associated **amyloid** beta-**peptide-binding protein** (**ERAB**) [Yan, Shi, Zhu, Fu, Zhu, Zhu, Gibson, Stern, Collison, Al-Mohanna et al. (1999) J. Biol. Chem. 274, 2145-2156]. Since human SCHAD and **ERAB** apparently possess

identical amino acid sequences, their catalytic properties should be identical. The recombinant SCHAD has been confirmed to be the right gene product and not a **mutant** variant. Steady-state kinetic measurements and quantitative analyses reveal that assay conditions such as pH and concentrations of coenzyme and substrate do not account for the kinetic differences reported for **ERAB** and SCHAD. Rather problematic experimental procedures appear to be responsible for the unrealistically high catalytic rate constants of **ERAB**. Eliminating the confusion surrounding the catalytic properties of this important multifunctional enzyme paves the way for exploring its role(s) in the pathogenesis of Alzheimer's disease.

L75 ANSWER 7 OF 17 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:691114 CAPLUS
DOCUMENT NUMBER: 131:318597
TITLE: The Alzheimer-associated .beta.-amyloid binding protein (**ERAB**) is highly upregulated in the testicular Leydig cells of the azoospermic W/Wv mouse: identification by differential display RT-PCR
INVENTOR(S): Ivell, Richard; Spiess, Andrej-Nikolei; Balvers, Marga; Jahner, Detlef; Hansis, Christoph
PATENT ASSIGNEE(S): Institut fur Hormon- und Fortpflanzungsforschung an der Universitat Hamburg, Germany
SOURCE: PCT Int. Appl., 40 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9954347	A2	19991028	WO 1999-EP2610	19990419
WO 9954347	A3	20000323		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9936070	A1	19991108	AU 1999-36070	19990419

PRIORITY APPLN. INFO.: US 1998-82257P P 19980417
WO 1999-EP2610 W 19990419

AB The present invention relates to a novel DDRT-PCR method of detecting genes expressed in tissues, esp. in **mutant** tissues such as murine wt./wt.v. The present invention also relates to novel genes detected by such method, including an Alzheimer-assocd. .beta.-amyloid protein or **ERAB**. Murine **ERAB** is a 260 amino acid polypeptide which is upregulated in the **mutant** testicular Leydig cells. Moderatly high homol. of this protein with the 3.alpha.,20.beta.-hydroxysteroid dehydrogenase from Streptomyces was found. The present invention also relates to a nucleic acid coding for such polypeptide, nucleic acids which hydridize under high stringency to such nucleic acid sequence, vectors, host cells, methods of expressing the nucleic acid sequence, and antibodies thereto. The nucleic acid and polypeptide are useful, e.g. as markers for testicular development.

L75 ANSWER 8 OF 17 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 1999107867 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9890977
TITLE: Role of **ERAB**/L-3-hydroxyacyl-coenzyme A dehydrogenase type II activity in Abeta-induced cytotoxicity.
AUTHOR: Yan S D; Shi Y; Zhu A; Fu J; Zhu H; Zhu Y; Gibson L; Stern E; Collison K; Al-Mohanna F; Ogawa S; Roher A; Clarke S G; Stern D M
CORPORATE SOURCE: Departments of Pathology, Physiology and Surgery, College of Physicians and Surgeons of Columbia University, New York, New York 10032, USA.

CONTRACT NUMBER: AG00690 (NIA)
AG11925 (NIA)
AG14103 (NIA)
SOURCE: Journal of biological chemistry, (1999 Jan 22) 274 (4) 2145-56.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990301
Last Updated on STN: 19990301
Entered Medline: 19990216

AB Endoplasmic reticulum-associated **amyloid** beta-**peptide** (Abeta)-**binding protein** (**ERAB**)/L-3-hydroxyacyl-CoA dehydrogenase type II (HADH II) is expressed at high levels in Alzheimer's disease (AD)-affected brain, binds Abeta, and contributes to Abeta-induced cytotoxicity. Purified recombinant **ERAB**/HADH II catalyzed the NADH-dependent reduction of S-acetoacetyl-CoA with a Km of approximately 68 microM and a Vmax of approximately 430 micromol/min/mg. The contribution of **ERAB**/HADH II enzymatic activity to Abeta-mediated cellular dysfunction was studied by site-directed **mutagenesis** in the catalytic domain (Y168G/K172G). Although COS cells cotransfected to overexpress wild-type **ERAB**/HADH II and variant beta-amyloid precursor protein (betaAPP(V717G)) showed DNA fragmentation, cotransfection with Y168G/K172G-altered **ERAB** and betaAPP(V717G) was without effect. We thus asked whether the enzyme might recognize alcohol substrates of which the aldehyde products could be cytotoxic; **ERAB**/HADH II catalyzed oxidation of a variety of simple alcohols (C2-C10) to their respective aldehydes in the presence of NAD+ and NAD-dependent oxidation of 17beta-estradiol. Addition of micromolar levels of synthetic Abeta(1-40) to purified **ERAB**/HADH II inhibited, in parallel, reduction of S-acetoacetyl-CoA (Ki approximately 1.6 microM), as well as oxidation of 17beta-estradiol (Ki approximately 3.2 microM) and (-)-2-octanol (Ki approximately 2.6 microM). Because micromolar levels of Abeta were required to inhibit **ERAB**/HADH II activity, whereas Abeta binding to **ERAB**/HADH II occurred at much lower concentrations (Km approximately 40-70 nM), the latter more closely simulating Abeta levels within cells, Abeta perturbation of **ERAB**/HADH II was likely to result from mechanisms other than the direct modulation of enzymatic activity. Cells cotransfected to overexpress **ERAB**/HADH II and betaAPP(V717G) generated malondialdehyde-protein and 4-hydroxynonenal-protein epitopes, which were detectable only at the lowest levels in cells overexpressing either **ERAB**/HADH II or betaAPP(V717G) alone. Generation of such toxic aldehydes was not observed in cells contransfected to overexpress Y168G/K172G-altered **ERAB** and betaAPP(V717G). We conclude that the generalized alcohol dehydrogenase activity of **ERAB**/HADH II is central to the cytotoxicity observed in an Abeta-rich environment.

L75 ANSWER 9 OF 17 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 1999:980684 SCISEARCH
THE GENUINE ARTICLE: 255MW
TITLE: The ER-associated body (**ERAB**), a novel membrane structure in yeast induced by **mutant** forms of Ste6p.
AUTHOR: Kuehn D L (Reprint); Nijbroek G L; Wright R; Michaelis S
CORPORATE SOURCE: JOHNS HOPKINS UNIV, SCH MED, BALTIMORE, MD 21205; UNIV WASHINGTON, SEATTLE, WA 98195
COUNTRY OF AUTHOR: USA
SOURCE: MOLECULAR BIOLOGY OF THE CELL, (NOV 1999) Vol. 10, Supp. [S], pp. 1237-1237.
Publisher: AMER SOC CELL BIOLOGY, PUBL OFFICE, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 1059-1524.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 0

L75 ANSWER 10 OF 17 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:62648 BIOSIS
DOCUMENT NUMBER: PREV200000062648
TITLE: The ER-associated body (**ERAB**), a novel membrane structure in yeast induced by **mutant** forms of Ste6p.
AUTHOR(S): Kuehn, Deborah L. [Reprint author]; Nijbroek, Gaby L. [Reprint author]; Wright, Robin; Michaelis, Susan [Reprint author]
CORPORATE SOURCE: Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD, USA
SOURCE: Molecular Biology of the Cell, (Nov., 1999) Vol. 10, No. SUPPL., pp. 213a. print.
Meeting Info.: 39th Annual Meeting of the American Society for Cell Biology. Washington, D.C., USA. December 11-15, 1999. The American Society for Cell Biology.
CODEN: MBCEEV. ISSN: 1059-1524.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 9 Feb 2000
Last Updated on STN: 3 Jan 2002

L75 ANSWER 11 OF 17 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1998252852 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9585418
TITLE: scully, an essential gene of Drosophila, is homologous to mammalian mitochondrial type II L-3-hydroxyacyl-CoA dehydrogenase/**amyloid**-beta **peptide-binding protein**.
AUTHOR: Torroja L; Ortuno-Sahagun D; Ferrus A; Hammerle B; Barbas J A
CORPORATE SOURCE: Instituto Cajal, Consejo Superior de Investigaciones Cientificas, 28002 Madrid, Spain.
SOURCE: Journal of cell biology, (1998 May 18) 141 (4) 1009-17.
Journal code: 0375356. ISSN: 0021-9525.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-Y15102
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 19980625
Last Updated on STN: 20000303
Entered Medline: 19980617

AB The characterization of scully, an essential gene of Drosophila with phenocritical phases at embryonic and pupal stages, shows its extensive homology with vertebrate type II L-3-hydroxyacyl-CoA dehydrogenase/**ERAB**. Genomic rescue demonstrates that four different lethal **mutations** are scu alleles, the molecular nature of which has been established. One of them, scu3127, generates a nonfunctional truncated product. scu4058 also produces a truncated protein, but it contains most of the known functional domains of the enzyme. The other two **mutations**, scu174 and scuS152, correspond to single amino acid changes. The expression of scully mRNA is general to many tissues including the CNS; however, it is highest in both embryonic gonadal primordia and mature ovaries and testes. Consistent with this pattern, the phenotypic analysis suggests a role for scully in germ line formation: **mutant** testis are reduced in size and devoid of maturing sperm, and **mutant** ovarioles are not able to produce viable eggs. Ultrastructural analysis of **mutant** spermatocytes reveals the presence of cytoplasmic lipid inclusions and scarce mitochondria. In addition, **mutant** photoreceptors contain morphologically aberrant mitochondria and large multilayered accumulations of membranous material. Some of these phenotypes are very similar to those present in human pathologies caused by beta-oxidation disorders.

L75 ANSWER 12 OF 17 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:51928 BIOSIS
DOCUMENT NUMBER: PREV199900051928

TITLE: Central role of **ERAB** enzymatic activity in Abeta-induced cytotoxicity in vitro.
AUTHOR(S): Yan, S. D. [Reprint author]; Shi, Y.; Zhu, A. [Reprint author]; Fu, J. [Reprint author]; Zhu, H. [Reprint author]; Zhu, Y. [Reprint author]; Gibson, L.; Roher, A.; Clarke, S.; Stern, D. [Reprint author]
CORPORATE SOURCE: Columbia Univ., New York, NY 10032, USA
SOURCE: Society for Neuroscience Abstracts, (1998) Vol. 24, No. 1-2, pp. 509. print.
Meeting Info.: 28th Annual Meeting of the Society for Neuroscience, Part 1. Los Angeles, California, USA. November 7-12, 1998. Society for Neuroscience.
ISSN: 0190-5295.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Slide)
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Feb 1999
Last Updated on STN: 10 Feb 1999

L75 ANSWER 13 OF 17 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 1999067016 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9851691
TITLE: The gene for the Alzheimer-associated beta-amyloid-binding protein (**ERAB**) is differentially expressed in the testicular Leydig cells of the azoospermic by w/w(v) mouse.
AUTHOR: Hansis C; Jahner D; Spiess A N; Boettcher K; Ivell R
CORPORATE SOURCE: IHF Institute for Hormone and Fertility Research, University of Hamburg, Germany.
SOURCE: European journal of biochemistry / FEBS, (1998 Nov 15) 258 (1) 53-60.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981228

AB In order to discover possible new testicular paracrine factors involved in the establishment of spermatogenesis, a modified differential display reverse transcription, polymerase chain reaction (DDRT-PCR) procedure was used to detect gene transcripts preferentially expressed in the testes of the azoospermic w/w(v) **mutant** mouse. One of the differentially expressed gene products showed partial similarity to members of the short-chain alcohol dehydrogenase family of enzymes. This cDNA fragment was used to obtain the full-length mouse cDNA sequence, which initially showed moderate similarity to a 20beta-steroid dehydrogenase from lower organisms, and later shown to have >85% similarity to a novel endoplasmic-reticulum-associated-binding protein (**ERAB**) from the human brain, implicated in Alzheimer's disease. A recently cloned bovine sequence also of high similarity suggests that this molecule might also represent an isozyme of 3-hydroxyacyl-CoA dehydrogenase. Using the mouse cDNA as probe, northern hybridization showed enrichment of the transcript to the testicular Leydig cells, and showed a specific approximately 20-fold enrichment in the azoospermic mouse testis. The level of the testicular **ERAB** transcript does not seem to change through puberty, suggesting that a lack of germ cells alone is not responsible for the up-regulation in the w/w(v) testis. Using the three-dimensional coordinates of the published 20beta-hydroxysteroid dehydrogenase structure as template, it was additionally possible to construct a molecular model of the novel protein which showed it to have a very similar structure to this enzyme, including the substrate-binding domain.

L75 ANSWER 14 OF 17 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 1998:76037 SCISEARCH
THE GENUINE ARTICLE: YQ995
TITLE: **Mutant** alleles of Ste6p and wild-type CFTR are retained in ''ER-associated bodies'' (**ERABs**) in Saccharomyces cerevisiae.

AUTHOR: Nijbroek G L (Reprint); Heinzer A; Michaelis S
CORPORATE SOURCE: JOHNS HOPKINS UNIV, SCH MED, DEPT CELL BIOL & ANAT, BALTIMORE, MD 21205
COUNTRY OF AUTHOR: USA
SOURCE: AMERICAN JOURNAL OF HUMAN GENETICS, (OCT 1997) Vol. 61, No. 4, Supp. [S], pp. 1501-1501.
Publisher: UNIV CHICAGO PRESS, 5720 S WOODLAWN AVE, CHICAGO, IL 60637.
ISSN: 0002-9297.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: English
REFERENCE COUNT: 0

L75 ANSWER 15 OF 17 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 97:119752 LIFESCI
TITLE: The ins and outs of amyloid- beta
AUTHOR: Beyreuther, K.; Masters, C.L.
CORPORATE SOURCE: ZMBH, Univ. Heidelberg, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany
SOURCE: NATURE, (19971000) vol. 389, no. 6652, pp. 677-678.
ISSN: 0028-0836.
DOCUMENT TYPE: Journal
TREATMENT CODE: General Review
FILE SEGMENT: N3
LANGUAGE: English
AB The devastating consequences of Alzheimer's disease--marked by extracellular senile plaques with fibrils of amyloid- beta peptide (A beta) and intraneuronal tangles of polymerized tau protein--are experienced by more and more elderly people in the Western world. The latest step towards understanding the underlying causes is reported by Yan et al. on page 689 of this issue. They have identified a possible mechanism to explain how A beta could damage neurons, and they show that A beta interacts with a hitherto unknown endoplasmic-reticulum-associated binding protein, **ERAB**. We already have compelling evidence that A beta may be at the root of neurodegeneration: **mutations** have been found in three genes--the amyloid precursor-protein (APP) gene on chromosome 21, and the genes for presenilin 1 (PS1) and presenilin 2 (PS2) on chromosomes 14 and 1, respectively--that segregate with rare forms of early-onset autosomal-dominant familial Alzheimer's disease. These **mutations** result in increased production of the APP cleavage product A beta 42, which is a main constituent of plaques but is not found in the paired helical filaments of tangles. Moreover, all of the **mutations** in the APP gene are within the A beta region. So although A beta seems to be implicated in neuropathogenesis and neurodegeneration, the missing link revolves around how it interferes with neuronal function and tangle formation.

L75 ANSWER 16 OF 17 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 97127408 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8972232
TITLE: Histone acetyltransferase activity is conserved between yeast and human GCN5 and is required for complementation of growth and transcriptional activation.
AUTHOR: Wang L; Mizzen C; Ying C; Candau R; Barlev N; Brownell J; Allis C D; Berger S L
CORPORATE SOURCE: Wistar Institute, Philadelphia, Pennsylvania 19104, USA.
SOURCE: Molecular and cellular biology, (1997 Jan) 17 (1) 519-27.
Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 20030204
Entered Medline: 19970122
AB Yeast and human ADA2 and GCN5 (y- and **hADA2** and y- and hGCN5, respectively) have been shown to potentiate transcription in vivo and may function as adaptors to bridge physical interactions between DNA-bound activators and the basal transcriptional machinery. Recently it was shown

that yGCN5 is a histone acetyltransferase (HAT), suggesting a link between enzymatic modification of nucleosomes and transcriptional activation. In this report, we demonstrate that hGCN5 is also an HAT and has the same substrate specificity as yGCN5. Since hGCN5 does not complement functional defects caused by deletion of yGCN5, we constructed a series of hGCN5-yGCN5 chimeras to identify human regions capable of activity in yeast. Interestingly, only the putative HAT domain of hGCN5, when fused to the remainder of yGCN5, complemented gcn5- cells for growth and transcriptional activation. Moreover, an amino acid substitution **mutation** within the HAT domain reduced both HAT activity in vitro and transcription in vivo. These findings directly link enzymatic histone acetylation and transcriptional activation and show evolutionary conservation of this potentially crucial pathway in gene regulation.

L75 ANSWER 17 OF 17 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:111422 BIOSIS
DOCUMENT NUMBER: PREV199800111422
TITLE: **Mutant** alleles of Ste6p and wild-type CFTR are retained in "ER-associated bodies" (**ERABs**) in Saccharomyces cerevisiae.
AUTHOR(S): Nijbroek, G. L.; Heinzer, A.; Michaelis, S.
CORPORATE SOURCE: John Hopkins Univ. Sch. Med., Dep. Cell Biol. Anatomy, Baltimore, MD 21205, USA
SOURCE: American Journal of Human Genetics, (Oct., 1997) Vol. 61, No. 4 SUPPL., pp. A258. print.
Meeting Info.: 47th Annual Meeting of the American Society of Human Genetics. Baltimore, Maryland, USA. October 28-November 1, 1997.
CODEN: AJHGAG. ISSN: 0002-9297.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Mar 1998
Last Updated on STN: 3 Mar 1998

=> log y

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L1          112 FILE MEDLINE
L2          214 FILE CAPLUS
L3          464 FILE SCISEARCH
L4           83 FILE LIFESCI
L5          128 FILE BIOSIS
L6          136 FILE EMBASE

TOTAL FOR ALL FILES
L7         1137 HIV-1 PROTEASE AND CRYSTAL

=> file medline

=> s hiv-1 protease and crystal
L8          112 HIV-1 PROTEASE AND CRYSTAL

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L9           26 L8 NOT 1994-2004/PY

=> d ibib abs 1-26

L9   ANSWER 1 OF 26     MEDLINE on STN
ACCESSION NUMBER:    94104010     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 8277496
TITLE:               Three-dimensional QSAR of human immunodeficiency virus (I)
                     protease inhibitors. 1. A CoMFA study employing
                     experimentally-determined alignment rules.
AUTHOR:              Waller C L; Oprea T I; Giolitti A; Marshall G R
CORPORATE SOURCE:    Center for Molecular Design, Washington University, St.
                     Louis, Missouri 63130.
CONTRACT NUMBER:     5 U01 AI 27302 (NIAID)
     GM 24483 (NIGMS)
     T32HL07275 (NHLBI)
SOURCE:              Journal of medicinal chemistry, (1993 Dec 24) 36 (26)
                     4152-60.
                     Journal code: 9716531. ISSN: 0022-2623.
PUB. COUNTRY:        United States
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals; AIDS
ENTRY MONTH:         199402
ENTRY DATE:          Entered STN: 19940218
                     Last Updated on STN: 19960129
                     Entered Medline: 19940204
AB   Comparative molecular field analysis (CoMFA), a three-dimensional,
     quantitative structure-activity relationship (QSAR) paradigm, was used to
     examine the correlations between the calculated physicochemical properties
     and the in vitro activities of a series of human immunodeficiency virus (
     HIV-1) protease inhibitors.  The training set
     consisted of 59 molecules from five structurally-diverse transition-state
     isostere classes: hydroxyethylamine, statine, norstatine, keto amide, and
     dihydroxyethylene.  The availability of X-ray crystallographic data for at
     least one representative from each class bound to the protease provided
     information regarding not only the active conformation of each ligand but
     also, via superimposition of protease backbones, the relative positions of
     each ligand with respect to one another in the active site of the enzyme.
     Once aligned, these molecules served as templates on which additional
     congeners were field-fit minimized.  Additional alignment rules were
     derived from minimizations of the ligands in the active site of the
     semirigid protease.  The predictive ability of each resultant model was
     evaluated using a test set comprised of molecules containing a novel
     transition-state isostere: hydroxyethylurea.  Crystallographic studies
     (Getman, D.P.; et al.  J.  Med.  Chem. 1993, 36, 288-291) indicated an
     unexpected binding mode for this series of compounds which precluded the
     use of the field-fit minimization alignment technique.  The test set
     molecules were, therefore, subjected to a limited systematic search in
     conjunction with active-site minimization.  The conformer of each molecule
     expressing the lowest interaction energy with the active site was included
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in the test set. Field-fit minimization of neutral molecules to **crystal** ligands and active-site minimizations of protonated ligands yielded predictive correlations for **HIV-1 protease** inhibitors. The use of crystallographic data in the determination of alignment rules and field-fit minimization as a molecular alignment tool in the absence of direct experimental data regarding binding modes is strongly supported by these results.

L9 ANSWER 2 OF 26 MEDLINE on STN
ACCESSION NUMBER: 94059982 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8241159
TITLE: Three-dimensional structure of a simian immunodeficiency virus protease/inhibitor complex. Implications for the design of human immunodeficiency virus type 1 and 2 protease inhibitors.
AUTHOR: Zhao B; Winborne E; Minnich M D; Culp J S; Debouck C; Abdel-Meguid S S
CORPORATE SOURCE: Department of Macromolecular Sciences, SmithKline Beecham, King of Prussia, Pennsylvania 19406.
SOURCE: Biochemistry, (1993 Dec 7) 32 (48) 13054-60.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940201
Last Updated on STN: 20000303
Entered Medline: 19940106

AB Simian immunodeficiency virus (SIV) proteins have considerable amino acid sequence homology to those from human immunodeficiency virus (HIV); thus monkeys are considered useful models for the preclinical evaluation of acquired immune deficiency syndrome (AIDS) therapeutics. We have crystallized and determined the three-dimensional structure of SIV protease bound to the hydroxyethylene isostere inhibitor SKF107457. **Crystals** of the complex were grown from 25-32% saturated sodium chloride, by the hanging drop method of vapor diffusion. They belong to the orthorhombic space group I222, with a = 46.3 A, b = 101.5 A, and c = 118.8 A. The structure has been determined at 2.5-A resolution by molecular replacement and refined to a crystallographic discrepancy factor, R (= sigma parallel Fo magnitude of - magnitude of Fc parallel/sigma magnitude of Fo magnitude of), of 0.189. The overall structure of the complex is very similar to previously reported structures of **HIV-1 protease** bound to inhibitors. The inhibitor is bound in a conformation that is almost identical to that found for the same inhibitor bound to **HIV-1 protease**, except for an overall translation of the inhibitor, varying along the backbone atoms from about 1.0 A at the termini to about 0.5 A around the scissile bond surrogate. The structures of the SIV and HIV-1 proteins vary significantly only in three surface loops composed of amino acids 15-20, 34-45, and 65-70. Superposition of the 1188 protein backbone atoms from the two structures gives an rms deviation of 1.0 A; this number is reduced to 0.6 A when atoms from the three surface loops are eliminated from the rms calculation.(ABSTRACT TRUNCATED AT 250 WORDS)

L9 ANSWER 3 OF 26 MEDLINE on STN
ACCESSION NUMBER: 93391360 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8378311
TITLE: **Crystal** structure of human immunodeficiency virus (HIV) type 2 protease in complex with a reduced amide inhibitor and comparison with **HIV-1 protease** structures.
AUTHOR: Tong L; Pav S; Pargellis C; Do F; Lamarre D; Anderson P C
CORPORATE SOURCE: Department of Medicinal Chemistry, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT 06877.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1993 Sep 15) 90 (18) 8387-91.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199310
ENTRY DATE: Entered STN: 19931105
Last Updated on STN: 20000303
Entered Medline: 19931020

AB The **crystal** structure of HIV-2 protease in complex with a reduced amide inhibitor [BI-LA-398; Phe-Val-Phe-psi (CH2NH)-Leu-Glu-Ile-amide] has been determined at 2.2-A resolution and refined to a crystallographic R factor of 17.6%. The rms deviation from ideality in bond lengths is 0.018 A and in bond angles is 2.8 degrees. The largest structural differences between HIV-1 and HIV-2 proteases are located at residues 15-20, 34-40, and 65-73, away from the flap region and the substrate binding sites. The rms distance between equivalent C alpha atoms of HIV-1 and HIV-2 protease structures excluding these residues is 0.5 A. The shapes of the S1 and S2 pockets in the presence of this inhibitor are essentially unperturbed by the amino acid differences between HIV-1 and HIV-2 proteases. The interaction of the inhibitor with HIV-2 protease is similar to that observed in **HIV-1 protease** structures. The unprotected N terminus of the inhibitor interacts with the side chains of Asp-29 and Asp-30. The glutamate side chain of the inhibitor forms hydrogen bonds with the main-chain amido groups of residues 129 and 130.

L9 ANSWER 4 OF 26 MEDLINE on STN
ACCESSION NUMBER: 93293821 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8514751
TITLE: The crystallographic structure of the protease from human immunodeficiency virus type 2 with two synthetic peptidic transition state analog inhibitors.
AUTHOR: Mulichak A M; Hui J O; Tomasselli A G; Heinrikson R L; Curry K A; Tomich C S; Thaisrivongs S; Sawyer T K; Watenpaugh K D
CORPORATE SOURCE: Discovery Research, Upjohn Company, Kalamazoo, Michigan 49007.
SOURCE: Journal of biological chemistry, (1993 Jun 25) 268 (18) 13103-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930806
Last Updated on STN: 20000303
Entered Medline: 19930722

AB The **crystal** structure of human immunodeficiency virus (HIV) type 2 protease has been determined in complexes with peptidic inhibitors Noa-His-Cha psi [CH(OH)CH(OH)]Val-Ile-Amp (U75875) and Qnc-Asn-Cha psi [CH(OH)CH2]Val-Npt(U92163) (where Noa is naphthyloxyacetyl, Cha is cyclohexylalanine, Amp is 2-aminomethylpyridine, Qnc is quinoline-2-carbonyl, and Npt is neopentylamine), which have dihydroxyethylene and hydroxyethylene moieties, respectively, in place of the normal scissile bond of the natural ligand. The complexes crystallize in space group P2(1)2(1)2(1), with one dimer-inhibitor complex per asymmetric unit and average cell dimensions of a = 33.28 A, b = 45.35 A, c = 135.84 A. Data were collected to approximately 2.5-A resolution. The model structures were refined with resulting R-factors of around 0.19. As expected, the HIV-2 protease structure is approximately C2-symmetric with a gross structure very similar to that of the HIV-1 enzyme. The inhibitors bind in an extended conformation positioned lengthwise in the binding cleft in a manner similar to that found in the **HIV-1 protease**-inhibitor complexes previously reported. The substitution of the bulkier Ile82 side chain in the HIV-2 protease may help explain the better ability of HIV-2 protease to bind and hydrolyze ligands with small P1 and P1' side groups. It appears that differences in specificity between the proteases of HIV-1 and HIV-2 are not merely a result of simple side chain substitutions, but may be complicated by differences in main chain flexibility as well.

L9 ANSWER 5 OF 26 MEDLINE on STN
ACCESSION NUMBER: 93278318 MEDLINE

DOCUMENT NUMBER: PubMed ID: 1304383
TITLE: **Crystal** structure of a complex of **HIV-1 protease** with a dihydroxyethylene-containing inhibitor: comparisons with molecular modeling.
AUTHOR: Thanki N; Rao J K; Foundling S I; Howe W J; Moon J B; Hui J O; Tomasselli A G; Heinrikson R L; Thaisrivongs S; Wlodawer A
CORPORATE SOURCE: Macromolecular Structure Laboratory, NCI-Frederick Cancer Research and Development Center, ABL-Basic Research Program, Maryland 21702.
CONTRACT NUMBER: NO1-CO-74101 (NCI)
SOURCE: Protein science : a publication of the Protein Society, (1992 Aug) 1 (8) 1061-72.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930716
Last Updated on STN: 19970203
Entered Medline: 19930708

AB The structure of a **crystal** complex of recombinant human immunodeficiency virus type 1 (**HIV-1**) **protease** with a peptide-mimetic inhibitor containing a dihydroxyethylene isostere insert replacing the scissile bond has been determined. The inhibitor is Noa-His-Hch psi [CH(OH)CH(OH)]Vam-Ile-Amp (U-75875), and its Ki for inhibition of the **HIV-1 protease** is < 1.0 nM (Noa = 1-naphthoxyacetyl, Hch = a hydroxy-modified form of cyclohexylalanine, Vam = a hydroxy-modified form of valine, Amp = 2-pyridylmethylamine). The structure of the complex has been refined to a crystallographic R factor of 0.169 at 2.0 A resolution by using restrained least-squares procedures. Root mean square deviations from ideality are 0.02 A and 2.4 degrees, for bond lengths and angles, respectively. The bound inhibitor diastereomer has the R configurations at both of the hydroxyl chiral carbon atoms. One of the diol hydroxyl groups is positioned such that it forms hydrogen bonds with both the active site aspartates, whereas the other interacts with only one of them. Comparison of this X-ray structure with a model-built structure of the inhibitor, published earlier, reveals similar positioning of the backbone atoms and of the side-chain atoms in the P2-P2' region, where the interaction with the protein is strongest. However, the X-ray structure and the model differ considerably in the location of the P3 and P3' end groups, and also in the positioning of the second of the two central hydroxyl groups. Reconstruction of the central portion of the model revealed the source of the hydroxyl discrepancy, which, when corrected, provided a P1-P1' geometry very close to that seen in the X-ray structure.

L9 ANSWER 6 OF 26 MEDLINE on STN
ACCESSION NUMBER: 93211902 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8460108
TITLE: Theoretical studies of relaxation of a monomeric subunit of **HIV-1 protease** in water using molecular dynamics.
AUTHOR: Venable R M; Brooks B R; Carson F W
CORPORATE SOURCE: Biophysics Laboratory, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892.
CONTRACT NUMBER: BBCA 1 R15 AI26307-01 (NIAID)
SOURCE: Proteins, (1993 Apr) 15 (4) 374-84.
Journal code: 8700181. ISSN: 0887-3585.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 19930514
Last Updated on STN: 19930514
Entered Medline: 19930423

AB The dynamic behavior of one 99-residue subunit of the dimeric aspartyl protease of HIV-1 was studied in a 160 psec molecular dynamics simulation

at 300 K in water. The **crystal** structure of one of the identical subunits of the dimer was the starting point, with the aqueous phase modeled by 4,331 explicit waters in a restrained spherical droplet. Analysis of the simulations showed that the monomer displayed considerable flexibility in the interfacial portions of the flap (the region which folds over the substrate), the N- and C-termini, and, to a lesser extent, the active site. The flap undergoes significant motion as an independent rigid finger, but without the cantilever previously reported in a simulation of the dimer. The N-terminus displayed the greatest fluctuational disorder whereas the C-terminus exhibited the greatest root mean square movement from the **crystal** structure. The central core of the monomer had a heavy-atom root mean square deviation from the initial structure of about 3.0 A during the latter half of the simulation. Although this is larger than the 1.6 A found for comparable simulations of typical globular proteins, the general features of the tertiary structure were preserved over the course of the simulation. Overall, these results indicate that the relaxed structure obtained in these simulations may provide a better model for the tertiary structure of the solvated **HIV-1 protease** monomer than the subunit conformation seen in the X-ray crystallographic structure of the dimer. Except in the flap region, the design of compounds intended to interfere with dimerization should take this relaxation and the flexibility of the solvated monomer, especially at the termini, into account.

L9 ANSWER 7 OF 26 MEDLINE on STN
ACCESSION NUMBER: 93160180 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8431424
TITLE: Molecular dynamics simulation of **HIV-1 protease** in a crystalline environment and in solution.
COMMENT: Erratum in: Biochemistry 1993 Mar 30;32(12):3196
AUTHOR: York D M; Darden T A; Pedersen L G; Anderson M W
CORPORATE SOURCE: Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709.
CONTRACT NUMBER: HL27995 (NHLBI)
SOURCE: Biochemistry, (1993 Feb 16) 32 (6) 1443-53.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930402
Last Updated on STN: 19970203
Entered Medline: 19930315

AB Simulations of the unbound form of the human immunodeficiency virus type 1 protease have been carried out to 200 ps in a crystalline environment and in solution. Solution simulations were performed with and without charge-balancing counterions. The results are compared with the 2.8-A crystallographic structure of Wlodawer et al. [(1989) Science 245, 616], and a proposed model for the solution structure which involves local refolding of the flap regions is presented. The simulations suggest the **crystal** packing environment of the protease dimer stabilizes the flaps in an extended conformation. Solvation of the dimer leads to local refolding of the flaps which contract toward the active site, forming increased overlap and stronger intersubunit hydrogn bonding at the tips. The degree to which the flaps overlap in solution is observed to depend on the charge state of the system.

L9 ANSWER 8 OF 26 MEDLINE on STN
ACCESSION NUMBER: 93136197 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8422397
TITLE: A symmetric inhibitor binds **HIV-1 protease** asymmetrically.
AUTHOR: Dreyer G B; Boehm J C; Chenera B; DesJarlais R L; Hassell A M; Meek T D; Tomaszek T A Jr; Lewis M
CORPORATE SOURCE: Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406.
CONTRACT NUMBER: GM-39526 (NIGMS)
SOURCE: Biochemistry, (1993 Jan 26) 32 (3) 937-47.

Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199302
ENTRY DATE: Entered STN: 19930312
Last Updated on STN: 19970203
Entered Medline: 19930225

AB Potential advantages of C2-symmetric inhibitors designed for the symmetric **HIV-1 protease** include high selectivity, potency, stability, and bioavailability. Pseudo-C2-symmetric monools and C2-symmetric diols, containing central hydroxymethylene and (R,R)-dihydroxyethylene moieties flanked by a variety of hydrophobic P1/P1' side chains, were studied as **HIV-1 protease** inhibitors. The monools and diols were synthesized in 8-10 steps from D-(+)-arabitol and D-(+)-mannitol, respectively. Monools with ethyl or isobutyl P1/P1' side chains were weak inhibitors of recombinant **HIV-1 protease** (Ki > 10 microM), while benzyl P1/P1' side chains afforded a moderately potent inhibitor (apparent Ki = 230 nM). Diols were 100-10,000x more potent than analogous monools, and a wider range of P1/P1' side chains led to potent inhibition. Both classes of compounds exhibited lower apparent Ki values under high-salt conditions. Surprisingly, monool and diol **HIV-1 protease** inhibitors were potent inhibitors of porcine pepsin, a prototypical asymmetric monomeric aspartic protease. These results were evaluated in the context of the pseudosymmetric structure of monomeric aspartic proteases and their evolutionary kinship with the retroviral proteases. The X-ray **crystal** structure of **HIV-1 protease** complexed with a symmetric diol was determined at 2.6 A. Contrary to expectations, the diol binds the protease asymmetrically and exhibits 2-fold disorder in the electron density map. Molecular dynamics simulations were conducted beginning with asymmetric and symmetric **HIV-1 protease** /inhibitor model complexes. A more stable trajectory resulted from the asymmetric complex, in agreement with the observed asymmetric binding mode. A simple four-point model was used to argue more generally that van der Waals and electrostatic force fields can commonly lead to an asymmetric association between symmetric molecules.

L9 ANSWER 9 OF 26 MEDLINE on STN
ACCESSION NUMBER: 92388948 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1517775
TITLE: An approach to computer-aided inhibitor design: application to cathepsin L.
AUTHOR: Sudarsanam S; Virca G D; March C J; Srinivasan S
CORPORATE SOURCE: Department of Protein Chemistry, Immunex Corporation, Seattle, WA 98101.
SOURCE: Journal of computer-aided molecular design, (1992 Jun) 6 (3) 223-33.
Journal code: 8710425. ISSN: 0920-654X.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199210
ENTRY DATE: Entered STN: 19921023
Last Updated on STN: 20020420
Entered Medline: 19921002

AB We have developed an approach to search for molecules that can be used as lead compounds in designing an inhibitor for a given proteolytic enzyme when the 3D structure of a homologous protein is known. This approach is based on taking the cast of the binding pocket of the protease and comparing its dimensions with that of the dimensions of small molecules. Herein the 3D structure of papain is used to model cathepsin L using the comparative modeling technique. The cast of the binding pocket is computed using the **crystal** structure of papain because the structures of papain and the model of cathepsin L are found to be similar at the binding site. The dimensions of the cast of the binding site of papain are used to screen for molecules from the Cambridge Structural Database (CSD) of small molecules. Twenty molecules out of the 80,000

small molecules in the CSD are found to have dimensions that are accommodated by the papain binding pocket. Visual comparison of the shapes of the cast and the 20 screened molecules resulted in identifying brevotoxin b, a toxin isolated from the 'red tide' dinoflagellate Ptycho brevis (previously classified as Gymonodium breve), as the structure that best fits the binding pocket of papain. We tested the proteolytic activity of papain and cathepsin L in the presence of brevotoxin b and found inhibition of papain and cathepsin L with Kis of 25 microM and 0.6 microM, respectively. We also compare our method with a more elaborate method in the literature, by presenting our results on the computer search for inhibitors of the **HIV-1 protease**.

L9 ANSWER 10 OF 26 MEDLINE on STN
ACCESSION NUMBER: 92345220 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1637805
TITLE: Hydroxyethylene isostere inhibitors of human immunodeficiency virus-1 protease: structure-activity analysis using enzyme kinetics, X-ray crystallography, and infected T-cell assays.
AUTHOR: Dreyer G B; Lambert D M; Meek T D; Carr T J; Tomaszek T A Jr; Fernandez A V; Bartus H; Cacciavillani E; Hassell A M; Minnich M; +
CORPORATE SOURCE: Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406.
CONTRACT NUMBER: GM-39526 (NIGMS)
SOURCE: Biochemistry, (1992 Jul 28) 31 (29) 6646-59.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920911
Last Updated on STN: 19970203
Entered Medline: 19920828

AB Analogues of peptides ranging in size from three to six amino acids and containing the hydroxyethylene dipeptide isosteres Phe psi Gly, Phe psi Ala, Phe psi NorVal, Phe psi Leu, and Phe psi Phe, where psi denotes replacement of CONH by (S)-CH(OH)CH2, were synthesized and studied as **HIV-1 protease** inhibitors. Inhibition constants (Ki) with purified **HIV-1 protease** depend strongly on the isostere in the order Phe psi Gly greater than Phe psi Ala greater than Phe psi NorVal greater than Phe psi Leu greater than Phe psi Phe and decrease with increasing length of the peptide analogue, converging to a value of 0.4 nM. Ki values are progressively less dependent on inhibitor length as the size of the P1' side chain within the isostere increases. The structures of **HIV-1 protease** complexed with the inhibitors Ala-Ala-X-Val-Val-OMe, where X is Phe psi Gly, Phe psi Ala, Phe psi NorVal, and Phe psi Phe, have been determined by X-ray crystallography (resolution 2.3-3.2 A). The **crystals** exhibit symmetry consistent with space group P6(1) with strong noncrystallographic 2-fold symmetry, and the inhibitors all exhibit 2-fold disorder. The inhibitors bind in similar conformations, forming conserved hydrogen bonds with the enzyme. The Phe psi Gly inhibitor adopts an altered conformation that places its P3' valine side chain partially in the hydrophobic S1' pocket, thus suggesting an explanation for the greater dependence of the Ki value on inhibitor length in the Phe psi Gly series. From the kinetic and crystallographic data, a minimal inhibitor model for tight-binding inhibition is derived in which the enzyme subsites S2-S2' are optimally occupied. The Ki values for several compounds are compared with their potencies as inhibitors of proteolytic processing in T-cell cultures chronically infected with HIV-1 (MIC values) and as inhibitors of acute infectivity (IC50 values). There is a rank-order correspondence, but a 20-1000-fold difference, between the values of Ki and those of MIC or IC50. IC50 values can approach those of Ki but are highly dependent on the conditions of the acute infectivity assay and are influenced by physiochemical properties of the inhibitors such as solubility.

L9 ANSWER 11 OF 26 MEDLINE on STN
ACCESSION NUMBER: 92294170 MEDLINE

DOCUMENT NUMBER: PubMed ID: 1603808
TITLE: Molecular dynamics of **HIV-1 protease**.
AUTHOR: Harte W E Jr; Swaminathan S; Beveridge D L
CORPORATE SOURCE: Chemistry Department, Hall-Atwater Laboratories, Wesleyan University, Middletown, Connecticut 06457.
CONTRACT NUMBER: GM 37909 (NIGMS)
SOURCE: Proteins, (1992 Jul) 13 (3) 175-94.
Journal code: 8700181. ISSN: 0887-3585.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 19920724
Last Updated on STN: 19920724
Entered Medline: 19920710

AB Molecular dynamics simulations have been carried out based on the GROMOS force field on the aspartyl protease (PR) of the human immunodeficiency virus HIV-1. The principal simulation treats the HIV-1 PR dimer and 6990 water molecules in a hexagonal prism cell under periodic boundary conditions and was carried out for a trajectory of 100 psec. Corresponding in vacuo simulations, i.e., treating the isolated protein without solvent, were carried out to study the influence of solvent on the simulation. The results indicate that including waters explicitly in the simulation results in a model considerably closer to the **crystal** structure than when solvent is neglected. Detailed conformational and helicoidal analysis was performed on the solvated form to determine the exact nature of the dynamical model and the exact points of agreement and disagreement with the **crystal** structure. The calculated dynamical model was further elucidated by means of studies of the time evolution of the cross-correlation coefficients for atomic displacements of the atoms comprising the protein backbone. The cross-correlation analysis revealed significant aspects of structure originating uniquely in the dynamical motions of the molecule. In particular, an unanticipated through-space, domain-domain correlation was found between the mobile flap region covering the active site and a remote regions of the structure, which collectively act somewhat like a molecular cantilever. The significance of these results is discussed with respect to the inactivation of the protease by site-specific mutagenesis, and in the design of inhibitors.

L9 ANSWER 12 OF 26 MEDLINE on STN
ACCESSION NUMBER: 92269238 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1588551
TITLE: Synthesis and antiviral activity of a series of **HIV -1 protease** inhibitors with functionality tethered to the P1 or P1' phenyl substituents: X-ray **crystal** structure assisted design.
AUTHOR: Thompson W J; Fitzgerald P M; Holloway M K; Emini E A; Darke P L; McKeever B M; Schleif W A; Quintero J C; Zugay J A; Tucker T J; +
CORPORATE SOURCE: Department of Medicinal Chemistry, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486.
SOURCE: Journal of medicinal chemistry, (1992 May 15) 35 (10) 1685-701.
Journal code: 9716531. ISSN: 0022-2623.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920710
Last Updated on STN: 19970203
Entered Medline: 19920623

AB By tethering of a polar hydrophilic group to the P1 or P1' substituent of a Phe-based hydroxyethylene isostere, the antiviral potency of a series of HIV protease inhibitors was improved. The optimum enhancement of anti-HIV activity was observed with the 4-morpholinylethoxy substituent. The substituent effect is consistent with a model derived from inhibitor

docked in the **crystal** structure of the native enzyme. An X-ray **crystal** structure of the inhibited enzyme determined to 2.25 A verifies the modeling predictions.

L9 ANSWER 13 OF 26 MEDLINE on STN
ACCESSION NUMBER: 92254733 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1812742
TITLE: The three-dimensional x-ray **crystal** structure of **HIV-1 protease** complexed with a hydroxyethylene inhibitor.
AUTHOR: Graves B J; Hatada M H; Miller J K; Graves M C; Roy S; Cook C M; Krohn A; Martin J A; Roberts N A
CORPORATE SOURCE: Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.
SOURCE: Advances in experimental medicine and biology, (1991) 306 455-60.
Journal code: 0121103. ISSN: 0065-2598.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920619
Last Updated on STN: 19920619
Entered Medline: 19920610

L9 ANSWER 14 OF 26 MEDLINE on STN
ACCESSION NUMBER: 92250592 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1315755
TITLE: Mutations that alter the activity of the Rous sarcoma virus protease.
AUTHOR: Grinde B; Cameron C E; Leis J; Weber I T; Wlodawer A; Burstein H; Bizub D; Skalka A M
CORPORATE SOURCE: Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106.
CONTRACT NUMBER: CA06927 (NCI)
CA38046 (NCI)
CA47486 (NCI)
+
SOURCE: Journal of biological chemistry, (1992 May 15) 267 (14) 9481-90.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920619
Last Updated on STN: 20000303
Entered Medline: 19920609

AB Mutations designed by analysis of the Rous sarcoma virus (RSV) and human immunodeficiency virus (**HIV**)-**1 protease** (PR) **crystal** structures were introduced into 1) the substrate binding pocket, 2) the substrate enclosing "flaps," and 3) surface loops of RSV PR. Each mutant PR was expressed in Escherichia coli. Changes in activity were detected by following cleavage of a truncated (NC-PR) precursor polypeptide in E. coli and cleavage of synthetic peptide substrates representing RSV and HIV-1 PR cleavage sites in vitro. Mutations in the substrate binding pocket exchanged amino acid residues located close to the substrate in the HIV-1 PR for structurally equivalent residues in the RSV PR. Changing histidine 65 to glycine (H65G) gave an inactive enzyme, while a double mutant R105P,G106V, as well as the triple mutant, H65G,R105P,G106V, produced enzymes which showed significant activity toward a substrate that represented a HIV-1 cleavage site. Mutating the catalytic aspartate (D37S) or an adjacent conserved alanine to threonine (A40T), produced inactive enzymes. In contrast, the substitution A40S was active, but showed a reduced rate of catalysis. Mutations in the flaps of conserved glycines (G69L, G70L) produced inactive PRs. Two extended RSV PR surface loops were shortened to the size found in HIV-1 PR and resulted in drastically reduced activity. These results have confirmed some of the basic predictions made from

structural models but have also revealed unexpected roles and interactions in the protein.

L9 ANSWER 15 OF 26 MEDLINE on STN
ACCESSION NUMBER: 91188281 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1707186
TITLE: **Crystal** structure of the ribonuclease H domain of HIV-1 reverse transcriptase.
COMMENT: Comment in: Science. 1991 Apr 5;252(5002):31. PubMed ID: 1849317
AUTHOR: Davies J F 2nd; Hostomska Z; Hostomsky Z; Jordan S R; Matthews D A
CORPORATE SOURCE: Agouron Pharmaceuticals, Inc., La Jolla, CA 92037.
CONTRACT NUMBER: GM 39599 (NIGMS)
SOURCE: Science, (1991 Apr 5) 252 (5002) 88-95.
Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199105
ENTRY DATE: Entered STN: 19910526
Last Updated on STN: 19970203
Entered Medline: 19910507

AB The **crystal** structure of the ribonuclease (RNase) H domain of HIV-1 reverse transcriptase (RT) has been determined at a resolution of 2.4 A and refined to a crystallographic R factor of 0.20. The protein folds into a five-stranded mixed beta sheet flanked by an asymmetric distribution of four alpha helices. Two divalent metal cations bind in the active site surrounded by a cluster of four conserved acidic amino acid residues. The overall structure is similar in most respects to the RNase H from Escherichia coli. Structural features characteristic of the retroviral protein suggest how it may interface with the DNA polymerase domain of p66 in the mature RT heterodimer. These features also offer insights into why the isolated RNase H domain is catalytically inactive but when combined in vitro with the isolated p51 domain of RT RNase H activity can be reconstituted. Surprisingly, the peptide bond cleaved by **HIV-1 protease** near the polymerase-RNase H junction of p66 is completely inaccessible to solvent in the structure reported here. This suggests that the homodimeric p66-p66 precursor of mature RT is asymmetric with one of the two RNase H domains at least partially unfolded.

L9 ANSWER 16 OF 26 MEDLINE on STN
ACCESSION NUMBER: 91129232 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1993177
TITLE: Structure at 2.5-A resolution of chemically synthesized human immunodeficiency virus type 1 protease complexed with a hydroxyethylene-based inhibitor.
AUTHOR: Jaskolski M; Tomasselli A G; Sawyer T K; Staples D G; Heinrikson R L; Schneider J; Kent S B; Wlodawer A
CORPORATE SOURCE: Macromolecular Structure Laboratory, National Cancer Institute-Frederick Cancer Research and Development Center, Maryland 21702-1201.
CONTRACT NUMBER: N01-CO-74101 (NCI)
SOURCE: Biochemistry, (1991 Feb 12) 30 (6) 1600-9.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199103
ENTRY DATE: Entered STN: 19910405
Last Updated on STN: 19970203
Entered Medline: 19910315

AB The **crystal** structure of a complex between chemically synthesized human immunodeficiency virus type 1 (**HIV-1**) **protease** and an octapeptide inhibitor has been refined to an R factor of 0.138 at 2.5-A resolution. The substrate-based inhibitor, H-Val-Ser-Gln-Asn-Leu psi [CH(OH)CH2]Val-Ile-Val-OH (U-85548e) contains a hydroxyethylene isostere replacement at the scissile bond that is believed

to mimic the tetrahedral transition state of the proteolytic reaction. This potent inhibitor has Ki less than 1 nM and was developed as an active-site titrant of the **HIV-1 protease**. The inhibitor binds in an extended conformation and is involved in beta-sheet interactions with the active-site floor and flaps of the enzyme, which form the substrate/inhibitor cavity. The inhibitor diastereomer has the S configuration at the chiral carbon atom of the hydroxyethylene insert, and the hydroxyl group is within H-bonding distance of the two active-site carboxyl groups in the enzyme dimer. The two subunits of the enzyme are related by a pseudodyad, which superposes them at a 178 degrees rotation. The main difference between the subunits is in the beta turns of the flaps, which have different conformations in the two monomers. The inhibitor has a clear preferred orientation in the active site and the alternative conformation, if any, is a minor one (occupancy of less than 30%). A new model of the enzymatic mechanism is proposed in which the proteolytic reaction is viewed as a one-step process during which the nucleophile (water molecule) and electrophile (an acidic proton) attack the scissile bond in a concerted manner.

L9 ANSWER 17 OF 26 MEDLINE on STN
ACCESSION NUMBER: 91062379 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2247458
TITLE: Domain communication in the dynamical structure of human immunodeficiency virus 1 protease.
AUTHOR: Harte W E Jr; Swaminathan S; Mansuri M M; Martin J C; Rosenberg I E; Beveridge D L
CORPORATE SOURCE: Chemistry Department, Hall-Atwater Laboratories, Wesleyan University, Middletown, CT 06457.
CONTRACT NUMBER: GM 37909 (NIGMS)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1990 Nov) 87 (22) 8864-8. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
OTHER SOURCE: PIR
ENTRY MONTH: 199101
ENTRY DATE: Entered STN: 19910222
Last Updated on STN: 19910222
Entered Medline: 19910110
AB A dynamical model for the structure of the human immunodeficiency virus 1 (**HIV-1**) **protease** dimer in aqueous solution has been developed on the basis of molecular dynamics simulation. The model provides an accurate account of the **crystal** geometry and also a prediction of the structural reorganization expected to occur in the protein in aqueous solution compared to the crystalline environment. Analysis of the results by means of dynamical cross-correlation coefficients for atomic displacements indicates that domain-domain communication is present in the protein in the form of a molecular "cantilever" and is likely to be involved in enzyme function at the molecular level. The dynamical structure also suggests information that may ultimately be useful in understanding and further development of specific inhibitors of **HIV-1 protease**.

L9 ANSWER 18 OF 26 MEDLINE on STN
ACCESSION NUMBER: 90370842 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2204060
TITLE: Structure-based design of nonpeptide inhibitors specific for the human immunodeficiency virus 1 protease.
AUTHOR: DesJarlais R L; Seibel G L; Kuntz I D; Furth P S; Alvarez J C; Ortiz de Montellano P R; DeCamp D L; Babe L M; Craik C S
CORPORATE SOURCE: Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco 94143-0446.
CONTRACT NUMBER: GM 13369 (NIGMS)
GM 31497 (NIGMS)
NIGMS-39552 (NIGMS)
+
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1990 Sep) 87 (17) 6644-8. Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199010
ENTRY DATE: Entered STN: 19901109
Last Updated on STN: 19970203
Entered Medline: 19901011

AB By using a structure-based computer-assisted search, we have found a butyrophenone derivative that is a selective inhibitor of the human immunodeficiency virus 1 (**HIV-1**) **protease**. The computer program creates a negative image of the active site cavity using the **crystal** structure of the **HIV-1** **protease**. This image was compared for steric complementarity with 10,000 molecules of the Cambridge Crystallographic Database. One of the most interesting candidates identified was bromperidol. Haloperidol, a closely related compound and known antipsychotic agent, was chosen for testing. Haloperidol inhibits the HIV-1 and HIV-2 proteases in a concentration-dependent fashion with a Ki of approximately 100 microM. It is highly selective, having little inhibitory effect on pepsin activity and no effect on renin at concentrations as high as 5 mM. The hydroxy derivative of haloperidol has a similar effect on **HIV-1** **protease** but a lower potency against the HIV-2 enzyme. Both haloperidol and its hydroxy derivative showed activity against maturation of viral polypeptides in a cell assay system. Although this discovery holds promise for the generation of nonpeptide protease inhibitors, we caution that the serum concentrations of haloperidol in normal use as an antipsychotic agent are less than 10 ng/ml (0.03 microM). Thus, concentrations required to inhibit the **HIV-1** **protease** are greater than 1000 times higher than the concentrations normally used. Haloperidol is highly toxic at elevated doses and can be life-threatening. Haloperidol is not useful as a treatment for AIDS but may be a useful lead compound for the development of an antiviral pharmaceutical.

L9 ANSWER 19 OF 26 MEDLINE on STN
ACCESSION NUMBER: 90354468 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2201691
TITLE: Specificity and inhibition of proteases from human immunodeficiency viruses 1 and 2.
AUTHOR: Tomasselli A G; Hui J O; Sawyer T K; Staples D J; Bannow C; Reardon I M; Howe W J; DeCamp D L; Craik C S; Heinrikson R L
CORPORATE SOURCE: Biopolymer Chemistry, Upjohn Company, Kalamazoo, Michigan 49001.
CONTRACT NUMBER: GM39552 (NIGMS)
SOURCE: Journal of biological chemistry, (1990 Aug 25) 265 (24) 14675-83.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901026
Last Updated on STN: 20020420
Entered Medline: 19900927

AB Highly purified, recombinant preparations of the virally encoded proteases from human immunodeficiency viruses (HIV) 1 and 2 have been compared relative to 1) their specificities toward non-viral protein and synthetic peptide substrates, and 2) their inhibition by several P1-P1' pseudodipeptidyl-modified substrate analogs. Hydrolysis of the Leu-Leu and Leu-Ala bonds in the Pseudomonas exotoxin derivative, Lys-PE40, is qualitatively the same for HIV-2 protease as published earlier for the HIV-1 enzyme (Tomasselli, A. G., Hui, J. O., Sawyer, T. K., Staples, D. J., FitzGerald, D. J., Chaudhary, V. K., Pastan, I., and Heinrikson, R. L. (1990) J. Biol. Chem. 265, 408-413). However, the rates of cleavage at these two sites are reversed for the HIV-2 protease which prefers the Leu-Ala bond. The kinetics of hydrolysis of this protein substrate by both enzymes are mirrored by those obtained from cleavage of model peptides. Hydrolysis by the two proteases of other synthetic peptides

modeled after processing sites in HIV-1 and HIV-2 gag polyproteins and selected analogs thereof demonstrated differences, as well as similarities, in selectivity. For example, while the two proteases were nearly identical in their rates of cleavage of the Tyr-Pro bond in the HIV-1 gag fragment, Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val, the **HIV-1 protease** showed a 64-fold enhancement over the HIV-2 enzyme in hydrolysis of a Tyr-Val bond in the same template. Accordingly, the HIV-2 protease appears to have a different specificity than the HIV-1 enzyme; it is better able to hydrolyze substrates with small amino acids in P1 and P1', but is variable in its rate of hydrolysis of peptides with bulky substituents in these positions. In addition to these comparisons of the two proteases with respect to substrate specificity, we present inhibitor structure-activity data for the HIV-2 protease. Relative to P1-P1' statine or Phe psi [CH2N]Pro-modified pseudopeptidyl inhibitors, compounds having Xaa psi[CH(OH)CH2]Yaa inserts were found to show significantly higher affinities to both enzymes, generally binding from 10 to 100 times stronger to **HIV-1 protease** than to the HIV-2 enzyme. Molecular modeling comparisons based upon the sequence homology of the two enzymes and x-ray **crystal** structures of **HIV-1 protease** suggest that most of the nonconservative amino acid replacements occur in regions well outside the catalytic cleft, while only subtle structural differences exist within the active site.(ABSTRACT TRUNCATED AT 400 WORDS)

L9 ANSWER 20 OF 26 MEDLINE on STN
ACCESSION NUMBER: 90354401 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2201682
TITLE: Crystallographic analysis of a complex between human immunodeficiency virus type 1 protease and acetyl-pepstatin at 2.0-A resolution.
AUTHOR: Fitzgerald P M; McKeever B M; VanMiddlesworth J F; Springer J P; Heimbach J C; Leu C T; Herber W K; Dixon R A; Darke P L
CORPORATE SOURCE: Department of Biophysical Chemistry, Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey 07065.
SOURCE: Journal of biological chemistry, (1990 Aug 25) 265 (24) 14209-19.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901026
Last Updated on STN: 20000303
Entered Medline: 19900927

AB The mode of binding of acetyl-pepstatin to the protease from the human immunodeficiency virus type 1 (HIV-1) has been determined by x-ray diffraction analysis. **Crystals** of an acetyl-pepstatin-**HIV-1 protease** complex were obtained in space group P2(1)2(1)2 (unit cell dimensions a = 58.39 A, b = 86.70 A, c = 46.27 A) by precipitation with sodium chloride. The structure was phased by molecular replacement methods, and a model for the structure was refined using diffraction data to 2.0 A resolution (R = 0.176 for 12901 reflections with I greater than sigma (I); deviation of bond distances from ideal values = 0.018 A; 172 solvent molecules included). The structure of the protein in the complex has been compared with the structure of the enzyme without the ligand. A core of 44 amino acids in each monomer, including residues in the active site and residues at the dimer interface, remains unchanged on binding of the inhibitor (root mean square deviation of alpha carbon positions = 0.39 A). The remaining 55 residues in each monomer undergo substantial rearrangement, with the most dramatic changes occurring at residues 44-57 (these residues comprise the so-called flaps of the enzyme). The flaps interact with one another and with the inhibitor so as to largely preserve the 2-fold symmetry of the protein. The inhibitor is bound in two approximately symmetric orientations. In both orientations the peptidyl backbone of the inhibitor is extended; a network of hydrogen bonds is formed between the inhibitor and the main body of the protein as well as between the inhibitor and the flaps. Hydrophobic side chains of residues in the body of the protein form partial binding sites for the side chains of the inhibitor;

hydrophobic side chains of residues in the flaps complete these binding sites.

L9 ANSWER 21 OF 26 MEDLINE on STN
ACCESSION NUMBER: 90353592 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2201571
TITLE: Comparison of inhibitor binding in **HIV-1 protease** and in non-viral aspartic proteases: the role of the flap.
AUTHOR: Gustchina A; Weber I T
CORPORATE SOURCE: Crystallography Laboratory, NCI-Frederick Cancer Research and Development Center, MD 21701.
CONTRACT NUMBER: N01-C01-74101
SOURCE: FEBS letters, (1990 Aug 20) 269 (1) 269-72.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901026
Last Updated on STN: 20000303
Entered Medline: 19900927
AB The **crystal** structure of **HIV-1 protease** with an inhibitor has been compared with the structures of non-viral aspartic proteases complexed with inhibitors. In the dimeric **HIV-1 protease**, two 4-stranded beta-sheets are formed by half of the inhibitor, residues 27-29, and the flap from each monomer. In the monomeric non-viral enzyme the single flap does not form a beta-sheet with an inhibitor. The **HIV-1 protease** shows more interactions with a longer peptide inhibitor than are observed in non-viral aspartic protease-inhibitor complexes. This, and the large movement of the flaps, restricts the conformation of the protease cleavage sites in the retroviral polyprotein precursor.

L9 ANSWER 22 OF 26 MEDLINE on STN
ACCESSION NUMBER: 90341771 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2200122
TITLE: Design, activity, and 2.8 A **crystal** structure of a C2 symmetric inhibitor complexed to **HIV-1 protease**.
AUTHOR: Erickson J; Neidhart D J; VanDrie J; Kempf D J; Wang X C; Norbeck D W; Plattner J J; Rittenhouse J W; Turon M; Wideburg N; +
CORPORATE SOURCE: Department of Computer-Assisted Molecular Design, Abbott Laboratories, Abbott Park, IL 60064.
CONTRACT NUMBER: AI 27220 (NIAID)
SOURCE: Science, (1990 Aug 3) 249 (4968) 527-33.
Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901012
Last Updated on STN: 20000303
Entered Medline: 19900912
AB A two-fold (C2) symmetric inhibitor of the protease of human immunodeficiency virus type-1 (HIV-1) has been designed on the basis of the three-dimensional symmetry of the enzyme active site. The symmetric molecule inhibited both protease activity and acute HIV-1 infection in vitro, was at least 10,000-fold more potent against **HIV-1 protease** than against related enzymes, and appeared to be stable to degradative enzymes. The 2.8 angstrom **crystal** structure of the inhibitor-enzyme complex demonstrated that the inhibitor binds to the enzyme in a highly symmetric fashion.

L9 ANSWER 23 OF 26 MEDLINE on STN
ACCESSION NUMBER: 90222119 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2158092
TITLE: Evaluation of homology modeling of HIV protease.

AUTHOR: Weber I T
CORPORATE SOURCE: Crystallography Laboratory, NCI-Frederick Cancer Research Facility, Maryland 21701.
CONTRACT NUMBER: N01-C01-74101
SOURCE: Proteins, (1990) 7 (2) 172-84.
Journal code: 8700181. ISSN: 0887-3585.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199005
ENTRY DATE: Entered STN: 19900622
Last Updated on STN: 20000303
Entered Medline: 19900514

AB The model of human immunodeficiency virus (**HIV-1**) **protease** which was based on the **crystal** structure of Rous sarcoma virus (RSV) protease has been compared to the recently determined **crystal** structure of chemically synthesized **HIV-1 protease**. The overall difference between the model and **crystal** structure was 1.4 A root mean square (rms) deviation for 86 superimposed C alpha atoms. The position of the flexible flap differs in the model and six residues at the amino terminus were incorrectly placed. With these exceptions, all atoms of the model and **crystal** structure agree to 2.1 A rms deviation. The conformation of some surface bends in the model agrees less well with the **crystal** structure. Identical amino acids in RSV and HIV proteases were modeled more reliably than different types of amino acids. The amino acids which form the substrate binding site were modeled most accurately to 1.2 A rms deviation for all atoms compared to the **crystal** structure. This suggests that functionally significant regions of related proteins can be modeled with high accuracy. The model gave correct predictions for residues making interactions with the substrate, and therefore could be used to design inhibitors. The model based on the RSV protease structure is more similar to the experimental structure than are previous models based on the structures of non-viral aspartic proteases.

L9 ANSWER 24 OF 26 MEDLINE on STN
ACCESSION NUMBER: 90220546 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2698993
TITLE: [Various aspects of structural studies of aspartate proteinases].
Nekotorye aspekty strukturnykh issledovanii aspartatnykh proteinaz.
AUTHOR: Andreeva N S; Gushchina A E; Zhdanov A S; Pechik I V; Safro M G; Fedorov A A
SOURCE: Molekuliarnaia biologiia, (1989 Nov-Dec) 23 (6) 1523-34.
Ref: 17
Journal code: 0105454. ISSN: 0026-8984.
PUB. COUNTRY: USSR
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199005
ENTRY DATE: Entered STN: 19900622
Last Updated on STN: 20000303
Entered Medline: 19900521

AB The paper is a brief account of aspartic proteinases' structural studies developed in V.A. Engelhardt Institute of Molecular Biology during the last 3 years. The work on porcine pepsin has been finalized after the refinement of the monoclinic **crystal** form at 1.8 A resolution performed in collaboration with the group of protein structure and function studies of the University of Alberta in Canada. An important structural property of chymosin which explains the enzyme specificity has been found. Protein engineering work on chymosin is being developed. The structural template for aspartic proteinases has been elucidated and on the basis of this template the model of **HIV-1 protease** molecule has been built. Some approaches to the design of **HIV-1 protease** inhibitors were elucidated.

L9 ANSWER 25 OF 26 MEDLINE on STN
ACCESSION NUMBER: 90121203 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2692557
TITLE: Crystallizable **HIV-1 protease** derived from expression of the viral pol gene in Escherichia coli.
AUTHOR: Danley D E; Geoghegan K F; Scheld K G; Lee S E; Merson J R; Hawrylik S J; Rickett G A; Ammirati M J; Hobart P M
CORPORATE SOURCE: Pfizer Central Research, Groton, CT 06340.
SOURCE: Biochemical and biophysical research communications, (1989 Dec 29) 165 (3) 1043-50.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199002
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 20000303
Entered Medline: 19900213

AB A plasmid vector was used to express the HIV-1 pol open reading frame under the regulation of the bacterial trp promoter in Escherichia coli. This expression system has been used as a source of recombinant viral protease. The self-processed active enzyme was recovered from a soluble fraction of a bacterial cell lysate and purified by a procedure involving four steps of chromatography. The protocol yielded 0.3 mg of protease for each liter of bacterial culture. The protease formed tetragonal bipyramidal **crystals** which have been used in high-resolution X-ray diffraction studies.

L9 ANSWER 26 OF 26 MEDLINE on STN
ACCESSION NUMBER: 89346747 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2548279
TITLE: Conserved folding in retroviral proteases: **crystal** structure of a synthetic **HIV-1 protease.**
AUTHOR: Wlodawer A; Miller M; Jaskolski M; Sathyanarayana B K; Baldwin E; Weber I T; Selk L M; Clawson L; Schneider J; Kent S B
CORPORATE SOURCE: Crystallography Laboratory, NCI-Frederick Cancer Research Facility, MD 21701.
CONTRACT NUMBER: N01-CO-74101 (NCI)
SOURCE: Science, (1989 Aug 11) 245 (4918) 616-21.
Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 198909
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 20000303
Entered Medline: 19890912

AB The rational design of drugs that can inhibit the action of viral proteases depends on obtaining accurate structures of these enzymes. The **crystal** structure of chemically synthesized **HIV-1 protease** has been determined at 2.8 angstrom resolution (R factor of 0.184) with the use of a model based on the Rous sarcoma virus protease structure. In this enzymatically active protein, the cysteines were replaced by alpha-amino-n-butyric acid, a nongenetically coded amino acid. This structure, in which all 99 amino acids were located, differs in several important details from that reported previously by others. The interface between the identical subunits forming the active protease dimer is composed of four well-ordered beta strands from both the amino and carboxyl termini and residues 86 to 94 have a helical conformation. The observed arrangement of the dimer interface suggests possible designs for dimerization inhibitors.

=> s cystein same (substituted or replaced) and crystal

MISSING OPERATOR 'SAME (SUBSTITUTE'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s cystein and (substituted or replaced) and crystal
L10 0 CYSTEIN AND (SUBSTITUTED OR REPLACED) AND CRYSTAL

=> s cysteine and (substituted or replaced) and crystal
L11 110 CYSTEINE AND (SUBSTITUTED OR REPLACED) AND CRYSTAL

=> s l11 not knot
L12 110 L11 NOT KNOT

=> s l12 not 2002-2004/py
L13 82 L12 NOT 2002-2004/PY

=> d 1-20 ibib abs

L13 ANSWER 1 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2001647862 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11700034
TITLE: **Crystal** structure of cobalt-containing nitrile hydratase.
AUTHOR: Miyanaga A; Fushinobu S; Ito K; Wakagi T
CORPORATE SOURCE: Department of Biotechnology, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.
SOURCE: Biochemical and biophysical research communications, (2001 Nov 16) 288 (5) 1169-74.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1IRE
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011112
Last Updated on STN: 20020123
Entered Medline: 20011226
AB The **crystal** structure of cobalt-containing nitrile hydratase from Pseudonocardia thermophila JCM 3095 at 1.8 A resolution revealed the structure of the noncorrin cobalt at the catalytic center. Two **cysteine** residues (alphaCys(111) and alphaCys(113)) coordinated to the cobalt were posttranslationally modified to **cysteine** -sulfinic acid and to **cysteine**-sulfenic acid, respectively, like in iron-containing nitrile hydratase. A tryptophan residue (betaTrp(72)), which may be involved in substrate binding, **replaced** the tyrosine residue of iron-containing nitrile hydratase. The difference seems to be responsible for the preference for aromatic nitriles rather than aliphatic ones of cobalt-containing nitrile hydratase.

L13 ANSWER 2 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2001555357 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 11601966
TITLE: Investigations of the Roles of the Distal Heme Environment and the Proximal Heme Iron Ligand in Peroxide Activation by Heme Enzymes via Molecular Engineering of Myoglobin.
AUTHOR: Ozaki Si; Roach M P; Matsui T; Watanabe Y
CORPORATE SOURCE: Institute for Molecular Science, and Department of Structural Molecular Science, The Graduate University for Advanced Studies, Myodaiji, Okazaki, 444-8585, Japan.
SOURCE: Accounts of chemical research, (2001 Oct) 34 (10) 818-25.
Journal code: 0157313. ISSN: 0001-4842.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-DATA-REVIEW; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20011017
Last Updated on STN: 20021211
AB To pursue structure-function relationships of heme enzymes in the activation of peroxides, we have chosen to use myoglobin as the framework

for our molecular engineering studies. Comparison of the **crystal** structures of myoglobin and peroxidases reveals differences in the arrangement of amino acid residues in heme active sites. On the basis of these structural differences and the reaction mechanisms of peroxidases, we have converted myoglobin into a peroxidase-like enzyme by alternation of the heme distal pocket via site-directed mutagenesis. The replacement of the proximal histidine with **cysteine** and the exogenous **substituted** imidazoles slightly accelerates the peroxide O-O bond cleavage due to the electron donor characteristics. However, we have not observed an enhancement in the activation of peroxide by the proximal mutant with tyrosine, the exogenous phenolate, and benzoate. A clear understanding of the absolute role of the proximal ligand remains elusive.

L13 ANSWER 3 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2001547055 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11485999
TITLE: alpha-Lactalbumin (LA) stimulates milk beta-1,4-galactosyltransferase I (beta 4Gal-T1) to transfer glucose from UDP-glucose to N-acetylglucosamine. **Crystal** structure of beta 4Gal-T1 x LA complex with UDP-Glc.
AUTHOR: Ramakrishnan B; Shah P S; Qasba P K
CORPORATE SOURCE: Structural Glycobiology Section, Laboratory of Experimental and Computational Biology, Center for Cancer Research, NCI-Frederick, National Institutes of Health, Frederick, Maryland 21702, USA.
CONTRACT NUMBER: NO1-CO-56000 (NCI)
SOURCE: Journal of biological chemistry, (2001 Oct 5) 276 (40) 37665-71.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1JN8; PDB-1JNA; PDB-1JNC
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011015
Last Updated on STN: 20030105
Entered Medline: 20011204
AB beta-1,4-Galactosyltransferase 1 (Gal-T1) transfers galactose (Gal) from UDP-Gal to N-acetylglucosamine (GlcNAc), which constitutes its normal galactosyltransferase (Gal-T) activity. In the presence of alpha-lactalbumin (LA), it transfers Gal to Glc, which is its lactose synthase (LS) activity. It also transfers glucose (Glc) from UDP-Glc to GlcNAc, constituting the glucosyltransferase (Glc-T) activity, albeit at an efficiency of only 0.3-0.4% of Gal-T activity. In the present study, we show that LA increases this activity almost 30-fold. It also enhances the Glc-T activity toward various N-acyl **substituted** glucosamine acceptors. Steady state kinetic studies of Glc-T reaction show that the K(m) for the donor and acceptor substrates are high in the absence of LA. In the presence of LA, the K(m) for the acceptor substrate is reduced 30-fold, whereas for UDP-Glc it is reduced only 5-fold. In order to understand this property, we have determined the **crystal** structures of the Gal-T1.LA complex with UDP-Glc x Mn(2+) and with N-butanoyl-glucosamine (N-butanoyl-GlcN), a preferred sugar acceptor in the Glc-T activity. The **crystal** structures reveal that although the binding of UDP-Glc is quite similar to UDP-Gal, there are few significant differences observed in the hydrogen bonding interactions between UDP-Glc and Gal-T1. Based on the present kinetic and **crystal** structural studies, a possible explanation for the role of LA in the Glc-T activity has been proposed.

L13 ANSWER 4 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2001431744 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11479344
TITLE: Molecular architecture of the voltage-dependent Na channel: functional evidence for alpha helices in the pore.
AUTHOR: Yamagishi T; Li R A; Hsu K; Marban E; Tomaselli G F
CORPORATE SOURCE: Institute of Molecular and Cellular Cardiology, Department of Medicine, The Johns Hopkins University, Baltimore, MD 21205, USA.
CONTRACT NUMBER: R01 HL50411 (NHLBI)

R01 HL52376 (NHLBI)
SOURCE: Journal of general physiology, (2001 Aug) 118 (2) 171-82.
Journal code: 2985110R. ISSN: 0022-1295.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200109
ENTRY DATE: Entered STN: 20011001
Last Updated on STN: 20011001
Entered Medline: 20010927

AB The permeation pathway of the Na channel is formed by asymmetric loops (P segments) contributed by each of the four domains of the protein. In contrast to the analogous region of K channels, previously we (Yamagishi, T., M. Janecki, E. Marban, and G. Tomaselli. 1997. Biophys. J. 73:195-204) have shown that the P segments do not span the selectivity region, that is, they are accessible only from the extracellular surface. The portion of the P-segment NH(2)-terminal to the selectivity region is referred to as SS1. To explore further the topology and functional role of the SS1 region, 40 amino acids NH(2)-terminal to the selectivity ring (10 in each of the P segments) of the rat skeletal muscle Na channel were **substituted** by **cysteine** and expressed in tsA-201 cells. Selected mutants in each domain could be blocked with high affinity by externally applied Cd(2)+ and were resistant to tetrodotoxin as compared with the wild-type channel. None of the externally applied sulfhydryl-specific methanethiosulfonate reagents modified the current through any of the mutant channels. Both R395C and R750C altered ionic selectivity, producing significant increases in K(+) and NH(4)(+) currents. The pattern of side chain accessibility is consistent with a pore helix like that observed in the **crystal** structure of the bacterial K channel, KcsA. Structure prediction of the Na channel using the program PHDhtm suggests an alpha helix in the SS1 region of each domain channel. We conclude that each of the P segments undergoes a hairpin turn in the permeation pathway, such that amino acids on both sides of the putative selectivity filter line the outer mouth of the pore. Evolutionary conservation of the pore helix motif from bacterial K channels to mammalian Na channels identifies this structure as a critical feature in the architecture of ion selective pores.

L13 ANSWER 5 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2001354878 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11300763
TITLE: Molecular motion of spin labeled side chains in alpha-helices: analysis by variation of side chain structure.
AUTHOR: Columbus L; Kalai T; Jeko J; Hideg K; Hubbell W L
CORPORATE SOURCE: Jules Stein Eye Institute and Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095-7008, USA.
CONTRACT NUMBER: EY05216 (NEI)
T3 EY07026 (NEI)
T32 GM08496 (NIGMS)
SOURCE: Biochemistry, (2001 Apr 3) 40 (13) 3828-46.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010625
Last Updated on STN: 20010625
Entered Medline: 20010621

AB Two single **cysteine** substitution mutants at helix surface sites in T4 lysozyme (D72C and V131C) have been modified with a series of nitroxide methanethiosulfonate reagents to investigate the structural and dynamical origins of their electron paramagnetic resonance spectra. The novel reagents include 4-**substituted** derivatives of either the pyrroline or pyrrolidine series of nitroxides. The spectral line shapes were analyzed as a function of side chain structure and temperature using a simulation method with a single order parameter and diffusion rates about three orthogonal axes as parameters. Taken together, the results

provide strong support for an anisotropic motional model of the side chain, which was previously proposed from qualitative features of the spectra and **crystal** structures of spin labeled T4 lysozyme. Site-specific differences in apparent order parameter are interpreted in terms of backbone dynamics modes with characteristic correlation times in the nanosecond or faster time scale. The saturated 4-**substituted** pyrrolidine nitroxides are shown to be a suitable template for novel "functionalized" side chains designed to mimic salient features of the native side chains they replace.

L13 ANSWER 6 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2001285227 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11327843
TITLE: Resonance energy transfer between tryptophan 57 in the epsilon subunit and pyrene maleimide labeled gamma subunit of the chloroplast ATP synthase.
AUTHOR: Johnson E A; Evron Y; McCarty R E
CORPORATE SOURCE: Department of Biology, Johns Hopkins University, Baltimore, MD 21218-2685, USA.
SOURCE: Biochemistry, (2001 Feb 13) 40 (6) 1804-11.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010529
Last Updated on STN: 20010529
Entered Medline: 20010524

AB The intrinsic fluorescence of the catalytic portion of the chloroplast ATP synthase (CF1) is quenched when **cysteine** 322, the penultimate amino acid of the gamma subunit, is specifically labeled with pyrene maleimide (PM). The epsilon subunit of CF1 contains the only two residues of tryptophan, which dominate the intrinsic fluorescence of unlabeled CF1. CF1 deficient in the epsilon subunit (CF1-epsilon) was reconstituted with mutant epsilon subunits in which phenylalanine **replaced** tryptophan at position 15 (epsilonW15F) and position 57 (epsilonW15/57F). CF1(epsilonW15F) containing a single tryptophan, epsilonW57, was labeled with PM at gammaC322. Resonance energy transfer (RET) from epsilonW57 to PM on gammaC322 occurred with an efficiency of energy transfer of 20%. RET was also observed from epsilonW57 to PM attached to the disulfide thiols of the gamma subunit (gammaC199,205) with an efficiency of approximately 45%. The R(o) (the distance at which the efficiency of energy transfer is 50%) for the epsilonW57 and PM donor/acceptor pair is 30 A, indicating that both gammaC322 and gammaC199,205 must be within 40 A of epsilonW57. These RET measurements show that both gammaC322 and gammaC199,205 are located near the base of the alpha/beta hexamer. This places the C-terminus of CF1 gamma much closer to epsilon than hypothesized based on homology to **crystal** structures of mitochondrial F1. These new RET measurements also allow the alignment of the predicted epsilon subunit structure. The orientation is similar to that predicted from cross-linking and mutational studies for the epsilon subunit of Escherichia coli F1.

L13 ANSWER 7 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2001121809 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11080639
TITLE: **Crystal** structure of the Escherichia coli peptide methionine sulphoxide reductase at 1.9 A resolution.
AUTHOR: Tete-Favier F; Cobessi D; Boschi-Muller S; Azza S; Branlant G; Aubry A
CORPORATE SOURCE: Laboratoire de Cristallographie et de Modelisation des Materiaux Mineraux et Biologiques Groupe Biocristallographie University Henri Poincare BP239 54506, Vandoeuvre-les-Nancy Cedex, France.
SOURCE: Structure with Folding & design, (2000 Nov 15) 8 (11) 1167-78.
Journal code: 100889329. ISSN: 0969-2126.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1FF3
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20021030
Entered Medline: 20010222

AB BACKGROUND: Peptide methionine sulphoxide reductases catalyze the reduction of oxidized methionine residues in proteins. They are implicated in the defense of organisms against oxidative stress and in the regulation of processes involving peptide methionine oxidation/reduction. These enzymes are found in numerous organisms, from bacteria to mammals and plants. Their primary structure shows no significant similarity to any other known protein. RESULTS: The X-ray structure of the peptide methionine sulphoxide reductase from Escherichia coli was determined at 3 A resolution by the multiple wavelength anomalous dispersion method for the selenomethionine-**substituted** enzyme, and it was refined to 1.9 A resolution for the native enzyme. The 23 kDa protein is folded into an alpha/beta roll and contains a large proportion of coils. Among the three **cysteine** residues involved in the catalytic mechanism, Cys-51 is positioned at the N terminus of an alpha helix, in a solvent-exposed area composed of highly conserved amino acids. The two others, Cys-198 and Cys-206, are located in the C-terminal coil. CONCLUSIONS: Sequence alignments show that the overall fold of the peptide methionine sulphoxide reductase from E. coli is likely to be conserved in many species. The characteristics observed in the Cys-51 environment are in agreement with the expected accessibility of the active site of an enzyme that reduces methionine sulphoxides in various proteins. Cys-51 could be activated by the influence of an alpha helix dipole. The involvement of the two other **cysteine** residues in the catalytic mechanism requires a movement of the C-terminal coil. Several conserved amino acids and water molecules are discussed as potential participants in the reaction.

L13 ANSWER 8 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2000476144 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11026676
TITLE: Structure-function studies of the non-heme iron active site of isopenicillin N synthase: some implications for catalysis.
AUTHOR: Kreisberg-Zakarin R; Borovok I; Yanko M; Frolow F; Aharonowitz Y; Cohen G
CORPORATE SOURCE: Department of Molecular Microbiology and Biotechnology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Israel.
SOURCE: Biophysical chemistry, (2000 Aug 30) 86 (2-3) 109-18. Journal code: 0403171. ISSN: 0301-4622.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010202

AB Isopenicillin N synthase (IPNS) is a non-heme ferrous iron-dependent oxygenase that catalyzes the ring closure of delta-(L-alpha-aminoadipoyl)-L-cysteinyl-D-valine (ACV) to form isopenicillin N. Spectroscopic studies and the **crystal** structure of IPNS show that the iron atom in the active species is coordinated to two histidine and one aspartic acid residues, and to ACV, dioxygen and H2O. We previously showed by site-directed mutagenesis that residues His212, Asp214 and His268 in the IPNS of Streptomyces jumonjinensis are essential for activity and correspond to the iron ligands identified by crystallography. To evaluate the importance of the nature of the protein ligands for activity, His214 and His268 were exchanged with asparagine, aspartic acid and glutamine, and Asp214 **replaced** with glutamic acid, histidine and **cysteine**, each of which has the potential to bind iron. Only the Asp214Glu mutant retained activity, approximately 1% that of the wild type. To determine the importance of the spatial arrangement of the protein ligands for activity, His212 and His268 were separately exchanged with Asp214; both mutant enzymes were completely defective. These

findings establish that IPNS activity depends critically on the presence of two histidine and one carboxylate ligands in a unique spatial arrangement within the active site. Molecular modeling studies of the active site employing the S. jumonjinensis IPNS **crystal** structure support this view. Measurements of iron binding by the wild type and the Asp214Glu, Asp214His and Asp214Cys-modified proteins suggest that Asp214 may have a role in catalysis as well as in iron coordination.

L13 ANSWER 9 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2000387719 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10884354
TITLE: Structure of a thioredoxin-like [2Fe-2S] ferredoxin from Aquifex aeolicus.
AUTHOR: Yeh A P; Chatelet C; Soltis S M; Kuhn P; Meyer J; Rees D C
CORPORATE SOURCE: Division of Chemistry and Chemical Engineering 147-75CH, California Institute of Technology, Pasadena, CA, 91125, USA.
SOURCE: Journal of molecular biology, (2000 Jul 14) 300 (3) 587-95. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1F37
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000818
Last Updated on STN: 20000818
Entered Medline: 20000810

AB The 2.3 A resolution **crystal** structure of a [2Fe-2S] cluster containing ferredoxin from Aquifex aeolicus reveals a thioredoxin-like fold that is novel among iron-sulfur proteins. The [2Fe-2S] cluster is located near the surface of the protein, at a site corresponding to that of the active-site disulfide bridge in thioredoxin. The four **cysteine** ligands are located near the ends of two surface loops. Two of these ligands can be **substituted** by non-native **cysteine** residues introduced throughout a stretch of the polypeptide chain that forms a protruding loop extending away from the cluster. The presence of homologs of this ferredoxin as components of more complex anaerobic and aerobic electron transfer systems indicates that this is a versatile fold for biological redox processes.

L13 ANSWER 10 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2000202045 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10736158
TITLE: Uphill electron transfer in the tetraheme cytochrome subunit of the Rhodopseudomonas viridis photosynthetic reaction center: evidence from site-directed mutagenesis.
AUTHOR: Chen I P; Mathis P; Koepke J; Michel H
CORPORATE SOURCE: Max-Planck-Institut fur Biophysik, Heinrich-Hoffmann-Strasse 7, D-60528 Frankfurt am Main, Germany.
SOURCE: Biochemistry, (2000 Apr 4) 39 (13) 3592-602. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000512
Last Updated on STN: 20000512
Entered Medline: 20000428

AB The cytochrome (cyt) subunit of the photosynthetic reaction center from Rhodopseudomonas viridis contains four heme groups in a linear arrangement in the spatial order heme1, heme2, heme4, and heme3. Heme3 is the direct electron donor to the photooxidized primary electron donor (special pair, P(+)). This heme has the highest redox potential (E(m)) among the hemes in the cyt subunit. The E(m) of heme3 has been specifically lowered by site-directed mutagenesis in which the Arg residue at the position of 264 of the cyt was **replaced** by Lys. The mutation decreases the E(m) of heme3 from +380 to +270 mV, i.e., below that of heme2 (+320 mV). In addition, a blue shift of the alpha-band was found to accompany the

mutation. The assignment of the lowered E(m) and the shifted alpha-band to heme3 was confirmed by spectroscopic measurements on RC **crystals**. The structure of the mutant RC has been determined by X-ray crystallography. No remarkable differences were found in the structure apart from the mutated residue itself. The velocity of the electron transfer (ET) from the tetraheme cyt to P(+) was measured under several redox conditions by following the rereduction of P(+) at 1283 nm after a laser flash. Heme3 donates an electron to P(+) with t(1/2) = 105 ns, i.e., faster than in the wild-type reaction center (t(1/2) = 190 ns), as expected from the larger driving force. The main feature is that a phase with t(1/2) approximately 2 micros dominates when heme3 is oxidized but heme2 is reduced. We conclude that the ET from heme2 to heme3 has a t(1/2) of approximately 2 micros, i.e., the same as in the WT, despite the fact that the reaction is endergonic by 50 meV instead of exergonic by 60 meV. We propose that the reaction kinetics is limited by the very uphill ET from heme2 to heme4, the DeltaG degrees of which is about the same (+230 meV) in both cases. The interpretation is further supported by measurements of the activation energy (216 meV in the wild-type, 236 meV in the mutant) and by approximate calculations of ET rates. Altogether these results demonstrate that the ET from heme2 to heme3 is stepwise, starting with a first very endergonic step from heme2 to heme4.

L13 ANSWER 11 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2000115460 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10648809
TITLE: Modification of Cys-418 of pyruvate formate-lyase by methacrylic acid, based on its radical mechanism.
AUTHOR: Plaga W; Vielhaber G; Wallach J; Knappe J
CORPORATE SOURCE: Biochemie-Zentrum Heidelberg (BZH), Ruprecht-Karls-Universitat, Im Neuenheimer Feld 501, 69120, Heidelberg, Germany.
SOURCE: FEBS letters, (2000 Jan 21) 466 (1) 45-8.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000309
Last Updated on STN: 20000309
Entered Medline: 20000222

AB The recently determined **crystal** structure of pyruvate formate-lyase (PFL) suggested a new view of the mechanism of this glycyl radical enzyme, namely that intermediary thiyl radicals of Cys-418 and Cys-419 participate in different ways [Becker, A. et al. (1999) Nat. Struct. Biol. 6, 969-975]. We report here a suicide reaction of PFL that occurs with the substrate-analog methacrylate with retention of the protein radical (K(I)=0.42 mM, k(i)=0.14 min(-1)). Using [1-(14)C]methacrylate (synthesized via acetone cyanhydrin), the reaction end-product was identified by peptide mapping and cocrystallization experiments as S-(2-carboxy-(2S)-propyl) **substituted** Cys-418. The stereoselectivity of the observed Michael addition reaction is compatible with a radical mechanism that involves Cys-418 thiyl as nucleophile and Cys-419 as H-atom donor, thus supporting the functional assignments of these catalytic amino acid residues derived from the protein structure.

L13 ANSWER 12 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2000092884 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10625656
TITLE: Structure and dynamics of the pore of inwardly rectifying K(ATP) channels.
AUTHOR: Loussouarn G; Makhina E N; Rose T; Nichols C G
CORPORATE SOURCE: Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110, USA.
CONTRACT NUMBER: HL54171 (NHLBI)
SOURCE: Journal of biological chemistry, (2000 Jan 14) 275 (2) 1137-44.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000309
Last Updated on STN: 20000309
Entered Medline: 20000218

AB Inwardly rectifying K(+) currents are generated by a complex of four Kir (Kir1-6) subunits. Pore properties are conferred by the second transmembrane domain (M2) of each subunit. Using cadmium ions as a **cysteine**-interacting probe, we examined the accessibility of **substituted cysteines** in M2 of the Kir6.2 subunit of inwardly rectifying K(ATP) channels. The ability of Cd(2+) ions to inhibit channels was used as the estimate of accessibility. The distribution of Cd(2+) accessibility is consistent with an alpha-helical structure of M2. The apparent surface of reactivity is broad, and the most reactive residues correspond to the solvent-accessible residues in the bacterial KcsA channel **crystal** structure. In several mutants, single channel measurements indicated that inhibition occurred by a single transition from the open state to a zero-conductance state. Analysis of currents expressed from mixtures of control and L164C mutant subunits indicated that at least three **cysteines** are required for coordination of the Cd(2+) ion. Application of phosphatidylinositol 4,5-diphosphate to inside-out membrane patches stabilized the open state of all mutants and also reduced cadmium sensitivity. Moreover, the Cd(2+) sensitivity of several mutants was greatly reduced in the presence of inhibitory ATP concentrations. Taken together, these results are consistent with state-dependent accessibility of single Cd(2+) ions to coordination sites within a relatively narrow inner vestibule.

L13 ANSWER 13 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2000022507 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10555581
TITLE: Characterization of metal-**substituted** Klebsiella aerogenes urease.
AUTHOR: Yamaguchi K; Cosper N J; Stalhandske C; Scott R A; Pearson M A; Karplus P A; Hausinger R P
CORPORATE SOURCE: Department of Microbiology, Michigan State University, East Lansing 48824-1101, USA.
CONTRACT NUMBER: DK45686 (NIDDK)
GM42025 (NIGMS)
SOURCE: Journal of biological inorganic chemistry : JBIC : a publication of the Society of Biological Inorganic Chemistry, (1999 Aug) 4 (4) 468-77.
Journal code: 9616326. ISSN: 0949-8257.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991217

AB Urease possesses a dinuclear Ni active site with the protein providing a bridging carbamylated lysine residue as well as an aspartyl and four histidyl ligands. The apoprotein can be activated in vitro by incubation with bicarbonate/CO2 and Ni(II); however, only approximately 15% forms active enzyme (Ni-CO2-ureaseA), with the remainder forming inactive carbamylated Ni-containing protein (Ni-CO2-ureaseB). In the absence of CO2, apoprotein plus Ni(II) forms a distinct inactive Ni-containing species (Ni-urease). The studies described here were carried out to better define the metal-binding sites for the inactive Ni-urease and Ni-CO2-ureaseB species, and to examine the properties of various forms of Co-, Mn-, and Cu-**substituted** ureases. Xray absorption spectroscopy (XAS) indicated that the two Ni atoms present in the Ni-urease metallocenter are coordinated by an average of two histidines and 3-4 N/O ligands, consistent with binding to the usual enzyme ligands with the lysine carbamate **replaced** by solvent. Neither XAS nor electronic spectroscopy provided evidence for thiolate ligation in the inactive Ni-containing species. By contrast, comparative studies of Co-CO2-urease and its C319A variant by electronic spectroscopy were

consistent with a portion of the two Co being coordinated by Cys319. Whereas the inactive Co-CO2-urease possesses a single histidyl ligand per metal, the species formed using C319A apoprotein more nearly resembles the native metallocenter and exhibits low levels of activity. Activity is also associated with one of two species of Mn-CO2-urease. A **crystal** structure of the inactive Mn-CO2-urease species shows a metallocenter very similar in structure to that of native urease, but with a disordering of the Asp360 ligand and movement in the Mn-coordinated solvent molecules. Cu(II) was bound to many sites on the protein in addition to the usual metallocenter, but most of the adventitious metal was removed by treatment with EDTA. Cu-treated urease was irreversibly inactivated, even in the C319A variant, and was not further characterized. Metal speciation between Ni, Co, and Mn most affected the higher of two pKa values for urease activity, consistent with this pKa being associated with the metal-bound hydrolytic water molecule. Our results highlight the importance of precisely positioned protein ligands and solvent structure for urease activity.

L13 ANSWER 14 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2000011395 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10542230
TITLE: Helix 4 of the Bacillus thuringiensis Cry1Aa toxin lines the lumen of the ion channel.
AUTHOR: Masson L; Tabashnik B E; Liu Y B; Brousseau R; Schwartz J L
CORPORATE SOURCE: National Research Council of Canada, Biotechnology Research Institute, Montreal, Quebec, Canada H4P 2R2..
Luke.Masson@Nrc.Ca
SOURCE: Journal of biological chemistry, (1999 Nov 5) 274 (45) 31996-2000.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991213

AB The mode of action of Bacillus thuringiensis insecticidal proteins is not well understood. Based on analogies with other bacterial toxins and ion channels, we hypothesized that charged amino acids in helix 4 of the Cry1Aa toxin are critical for toxicity and ion channel function. Using Plutella xylostella as a model target, we analyzed responses to Cry1Aa and eight proteins with altered helix 4 residues. Toxicity was abolished in five charged residue mutants (E129K, R131Q, R131D, D136N, D136C), however, two charged (R127E and R127N) and one polar (N138C) residue mutant retained wild-type toxicity. Compared with Cry1Aa and toxic mutants, nontoxic mutants did not show greatly reduced binding to brush border membrane vesicles, but their ion channel conductance was greatly reduced in planar lipid bilayers. **Substituted cysteine** accessibility tests showed that in situ restoration of the negative charge of D136C restored conductance to wild-type levels. The results imply that charged amino acids on the Asp-136 side of helix 4 are essential for toxicity and passage of ions through the channel. These results also support a refined version of the umbrella model of membrane integration in which the side of helix 4 containing Asp-136 faces the aqueous lumen of the ion channel.

L13 ANSWER 15 OF 82 MEDLINE on STN
ACCESSION NUMBER: 2000002663 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10531337
TITLE: Oxidation of the alpha(3)(betaD311C/R333C)(3)gamma subcomplex of the thermophilic Bacillus PS3 F(1)-ATPase indicates that only two beta subunits can exist in the closed conformation simultaneously.
AUTHOR: Ren H; Dou C; Stelzer M S; Allison W S
CORPORATE SOURCE: Department of Chemistry, University of California at San Diego, La Jolla, California 92093-0506, USA.
CONTRACT NUMBER: GM16974 (NIGMS)
SOURCE: Journal of biological chemistry, (1999 Oct 29) 274 (44) 31366-72.

Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991216

AB In the **crystal** structure of the bovine heart mitochondrial F(1)-ATPase (Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994) Nature 370, 621-628), the two liganded beta subunits, one with MgAMP-PNP bound to the catalytic site (beta(T)) and the other with MgADP bound (beta(D)) have closed conformations. The empty beta subunit (beta(E)) has an open conformation. In beta(T) and beta(D), the distance between the carboxylate of beta-Asp(315) and the guanidinium of beta-Arg(337) is 3.0-4.0 A. These side chains are at least 10 A apart in beta(E). The alpha(3)(betaD311C/R333C)(3)gamma subcomplex of TF(1) with the corresponding residues **substituted** with **cysteine** has very low ATPase activity unless it is reduced prior to assay or assayed in the presence of dithiothreitol. The reduced subcomplex hydrolyzes ATP at 50% the rate of wild-type and is rapidly inactivated by oxidation by CuCl(2) with or without magnesium nucleotides bound to catalytic sites. Titration of the subcomplex with iodo[(14)C]acetamide after prolonged treatment with CuCl(2) in the presence or absence of 1 mM MgADP revealed nearly two free sulfhydryl groups/mol of enzyme. Therefore, one pair of introduced **cysteines** is located on a beta subunit that exists in the open or partially open conformation even when catalytic sites are saturated with MgADP. Since V(max) of ATP hydrolysis is attained when three catalytic sites of F(1) are saturated, the catalytic site that binds ATP must be closing as the catalytic site that releases products is opening.

L13 ANSWER 16 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1999234347 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10216292
TITLE: Rubredoxin from Clostridium pasteurianum. Structures of G10A, G43A and G10VG43A mutant proteins. Mutation of conserved glycine 10 to valine causes the 9-10 peptide link to invert.
AUTHOR: Maher M J; Xiao Z; Wilce M C; Guss J M; Wedd A G
CORPORATE SOURCE: School of Chemistry, University of Melbourne, Parkville, Victoria, 3052, Australia.
SOURCE: Acta crystallographica. Section D, Biological crystallography, (1999 May) 55 (5) 962-8.
Journal code: 9305878. ISSN: 0907-4449.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1B13; PDB-1B20; PDB-1B2J
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990714
Last Updated on STN: 19990714
Entered Medline: 19990630

AB The four **cysteine** ligands which coordinate the Fe atom in the electron-transfer protein rubredoxin lie on loops of the polypeptide which form approximate local twofold symmetry. The **cysteine** ligands in the protein from Clostridium pasteurianum lie at positions 6, 9, 39 and 42. Two glycine residues adjacent to the **cysteine** ligands at positions 10 and 43 are conserved in all rubredoxins, consistent with the proposal that a beta-carbon substituent at these positions would eclipse adjacent peptide carbonyl groups [Adman et al. (1975). Proc. Natl Acad. Sci. USA, 72, 4854-4858]. X-ray **crystal** structures of the three mutant proteins G10A, G43A and G10VG43A are reported. The **crystal** structures of the single-site mutations are isomorphous with the native protein, space group R3; unit-cell parameters are a = 64.3, c = 32.9 A for G10A and a = 64.4, c = 32.8 A for G43A. The **crystals** of the double mutant, G10VG43A, were in space group P43212, unit-cell parameters a = 61.9, c = 80.5 A, with two molecules per asymmetric unit. The observed structural perturbations support the

hypothesis that mutation of the conserved glycine residues would introduce strain into the polypeptide. In particular, in the G10VG43A protein substitution of valine at Gly10 causes the 9-10 peptide link to invert, relieving steric interaction between Cys9 O and Val10 Cbeta. This dramatic change in conformation is accompanied by the loss of the 10N-HcO6 hydrogen bond, part of the chelate loop Thr5-Tyr11. The new conformation allows retention of the 11N-HcS9 hydrogen bond, but converts it from a type II to a type I hydrogen bond. This occurs at the cost of a less tightly packed structure. The structural insights allow rationalization of 1H NMR data reported previously for the 113CdII-**substituted** proteins and of the negative shifts observed in the FeIII/FeII mid-point potentials upon mutation.

L13 ANSWER 17 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1999190957 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10089479
TITLE: Separation and crystallization of T = 3 and T = 4 icosahedral complexes of the hepatitis B virus core protein.
AUTHOR: Zlotnick A; Palmer I; Kaufman J D; Stahl S J; Steven A C; Wingfield P T
CORPORATE SOURCE: Protein Expression Laboratory, National -Institute of Arthritis, Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892, USA.
SOURCE: Acta crystallographica. Section D, Biological crystallography, (1999 Mar) 55 (Pt 3) 717-20. Journal code: 9305878. ISSN: 0907-4449.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990504
Last Updated on STN: 20000303
Entered Medline: 19990419

AB The icosahedral nucleocapsid of human hepatitis B virus is a homopolymer of the dimeric capsid protein also known as hepatitis B core antigen or HBcAg. Purified capsid protein obtained from an Escherichia coli expression system was reassembled into a mixture of T = 3 and T = 4 icosahedral particles consisting of 90 and 120 dimers, respectively. The two types of capsid were separated on a preparative scale by centrifugation through a sucrose gradient. In addition to this heterogeneity, the capsid protein has three **cysteines**, one of which has a great propensity for forming disulfide bonds between the two subunits, forming a dimer. To eliminate heterogeneity arising from oxidation, alanines were **substituted** for the **cysteines** . T = 3 and T = 4 capsids crystallized under similar conditions. **Crystals** of T = 3 capsids diffracted to approximately 8 A resolution; **crystals** of T = 4 capsids diffracted to 4 A resolution.

L13 ANSWER 18 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1999017901 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9799508
TITLE: N-termini of EcoRI restriction endonuclease dimer are in close proximity on the protein surface.
AUTHOR: Liu W; Chen Y; Watrob H; Bartlett S G; Jen-Jacobson L; Barkley M D
CORPORATE SOURCE: Department of Chemistry, Louisiana State University, Baton Rouge 70803, USA.
CONTRACT NUMBER: GM29207 (NIGMS)
GM35009 (NIGMS)
SOURCE: Biochemistry, (1998 Nov 3) 37 (44) 15457-65. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20000303

Entered Medline: 19981130

AB The N-terminal region of EcoRI endonuclease is essential for cleavage yet is invisible in the 2.5 A **crystal** structure of endonuclease-DNA complex [Kim, Y., Grable, J. C., Love, R., Greene, P. J., Rosenberg, J. M. (1990) Science 249, 1307-1309]. We used site-directed fluorescence spectroscopy and chemical cross-linking to locate the N-terminal region and assess its flexibility in the absence and presence of DNA substrate. The second amino acid in each subunit of the homodimer was **replaced** with **cysteine** and labeled with pyrene or reacted with bifunctional cross-linkers. The broad absorption spectra and characteristic excimer emission bands of pyrene-labeled muteins indicated stacking of the two pyrene rings in the homodimer. Proximity of N-terminal **cysteines** was confirmed by disulfide bond formation and chemical cross-linking. The dynamics of the N-terminal region were determined from time-resolved emission anisotropy measurements. The anisotropy decay had two components: a fast component with rotational correlation time of 0.3-3 ns representing probe internal motions and a slow component with 50-100 ns correlation time representing overall tumbling of the protein conjugate. We conclude that the N-termini are close together at the dimer interface with limited flexibility. Binding of Mg2+ cofactor or DNA substrate did not affect the location or flexibility of the N-terminal region as sensed by pyrene fluorescence and cross-linking, indicating that substrate binding is not accompanied by folding or unfolding of the N-terminus.

L13 ANSWER 19 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1998440449 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9767629
TITLE: Structure-based design of cathepsin K inhibitors containing a benzyloxy-**substituted** benzoyl peptidomimetic.
AUTHOR: Thompson S K; Smith W W; Zhao B; Halbert S M; Tomaszek T A; Tew D G; Levy M A; Janson C A; DAlessio K J; McQueney M S; Kurdyla J; Jones C S; DesJarlais R L; Abdel-Meguid S S; Veber D F
CORPORATE SOURCE: Departments of Medicinal Chemistry, Structural Biology, Molecular Recognition, Protein Biochemistry, and Physical and Structural Chemistry, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406, USA.
SOURCE: Journal of medicinal chemistry, (1998 Oct 8) 41 (21) 3923-7.
Journal code: 9716531. ISSN: 0022-2623.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1AYU; PDB-1AYW
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981103

AB Peptidomimetic cathepsin K inhibitors have been designed using binding models which were based on the X-ray **crystal** structure of an amino acid-based, active site-spanning inhibitor complexed with cathepsin K. These inhibitors, which contain a benzyloxybenzoyl group in place of a Cbz-leucine moiety, maintained good inhibitory potency relative to the amino acid-based inhibitor, and the binding models were found to be very predictive of relative inhibitor potency. The binding mode of one of the inhibitors was confirmed by X-ray crystallography, and the crystallographically determined structure is in close qualitative agreement with the initial binding model. These results strengthen the validity of a strategy involving iterative cycles of structure-based design, inhibitor synthesis and evaluation, and crystallographic structure determination for the discovery of peptidomimetic inhibitors.

L13 ANSWER 20 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1998437492 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9761825
TITLE: Crystallization and preliminary crystallographic study of a component of the Escherichia coli tol system: TolB.
AUTHOR: Abergel C; Rigal A; Chenivesse S; Lazdunski C; Claverie J M; Bouveret E; Benedetti H

CORPORATE SOURCE: Information Genetique et Structurale, EP 91, Institut de Biologie Structurale et Microbiologie, 31 Chemin Joseph Aiguier, Marseille, 13402 CEDEX 20, France.. chantal@igs.cnrs-mrs.fr
SOURCE: Acta crystallographica. Section D, Biological crystallography, (1998 Jan 1) 54 (Pt 1) 102-4. Journal code: 9305878. ISSN: 0907-4449.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 20020723
Entered Medline: 19981130

AB TolB from Escherichia coli is part of the Tol system used by the group A colicins to penetrate and kill cells. A TolB derivative tagged with six histidines was overexpressed, purified by chelation on a nickel affinity column and crystallized using the SAmBA software to define the optimal crystallization protocol. The **crystals** belong to the monoclinic system, space group P21 with unit-cell parameters a = 64.48, b = 41.06, c = 78.41 A, beta = 110.78 degrees. Frozen **crystals** diffract to 1.9 A resolution. Screening for heavy-atom derivatives both on the native TolB and various **cysteine-substituted** mutants is in progress. In addition, a selenomethionine-**substituted** protein is being produced in order to use the MAD method for structure determination.

=> d 21-82 ibib abs

L13 ANSWER 21 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1998361241 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9697776
TITLE: Structure of the metal-ion-activated diphtheria toxin repressor/tox operator complex.
AUTHOR: White A; Ding X; vanderSpek J C; Murphy J R; Ringe D
CORPORATE SOURCE: Rosenstiel Basic Medical Sciences Research Center MS029, Brandeis University, Waltham, Massachusetts 02454-9110, USA.
SOURCE: Nature, (1998 Jul 30) 394 (6692) 502-6. Journal code: 0410462. ISSN: 0028-0836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1DDN; PDB-2TDX
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 19980820
Last Updated on STN: 19980820
Entered Medline: 19980813

AB The virulent phenotype of the pathogenic bacterium Corynebacterium diphtheriae is conferred by diphtheria toxin, whose expression is an adaptive response to low concentrations of iron. The expression of the toxin gene (tox) is regulated by the repressor DtxR, which is activated by transition metal ions. X-ray **crystal** structures of DtxR with and without (apo-form) its coordinated transition metal ion have established the general architecture of the repressor, identified the location of the metal-binding sites, and revealed a metal-ion-triggered subunit-subunit 'caliper-like' conformational change. Here we report the three-dimensional **crystal** structure of the complex between a biologically active Ni(II)-bound DtxR(C102D) mutant, in which a **cysteine** is **replaced** by an aspartate at residue 102, and a 33-base-pair DNA segment containing the toxin operator toxO. This structure shows that DNA interacts with two dimeric repressor proteins bound to opposite sides of the tox operator. We propose that a metal-ion-induced helix-to-coil structural transition in the amino-terminal region of the protein is partly responsible for the unique mode of repressor activation by transition metal ions.

L13 ANSWER 22 OF 82 MEDLINE on STN

ACCESSION NUMBER: 1998352055 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9685368
TITLE: **Crystal** structures of Flavobacterium glycosylasparaginase. An N-terminal nucleophile hydrolase activated by intramolecular proteolysis.
AUTHOR: Guo H C; Xu Q; Buckley D; Guan C
CORPORATE SOURCE: Department of Biophysics, Boston University School of Medicine, Boston, Massachusetts 02118-2526, USA.. hguo@med-biophd.bu.edu
SOURCE: Journal of biological chemistry, (1998 Aug 7) 273 (32) 20205-12.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-2GAC; PDB-2GAW
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980917
Last Updated on STN: 19980917
Entered Medline: 19980910

AB Glycosylasparaginase (GA) is a member of a novel family of N-terminal nucleophile hydrolases that catalytically use an N-terminal residue as both a polarizing base and a nucleophile. These enzymes are activated from a single chain precursor by intramolecular autoproteolysis to yield the N-terminal nucleophile. A deficiency of GA results in the human genetic disorder known as aspartylglycosaminuria. In this study, we report the **crystal** structure of recombinant GA from Flavobacterium meningosepticum. Similar to the human structure, the bacterial GA forms an alphabetabetaalpha sandwich. However, some significant differences are observed between the Flavobacterium and human structures. The active site of Flavobacterium glycosylasparaginase is in an open conformation when compared with the human structure. We also describe the structure of a mutant wherein the N-terminal nucleophile Thr152 is **substituted** by a **cysteine**. In the bacterial GA **crystals**, we observe a heterotetrameric structure similar to that found in the human structure, as well as that observed in solution for eukaryotic glycosylasparaginases. The results confirm the suitability of the bacterial enzyme as a model to study the consequences of mutations in aspartylglycosaminuria patients. They also suggest that further studies are necessary to understand the detail mechanism of this enzyme. The presence of the heterotetrameric structure in the **crystals** is significant because dimerization of precursors has been suggested in the human enzyme to be a prerequisite to trigger autoproteolysis.

L13 ANSWER 23 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1998308000 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9642075
TITLE: Design, synthesis and structure of a zinc finger with an artificial beta-turn.
AUTHOR: Viles J H; Patel S U; Mitchell J B; Moody C M; Justice D E; Uppenbrink J; Doyle P M; Harris C J; Sadler P J; Thornton J M
CORPORATE SOURCE: Department of Chemistry, Birkbeck College, University of London, Gordon House, London, WC1H 0PP, UK.
SOURCE: Journal of molecular biology, (1998 Jun 19) 279 (4) 973-86.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980731
Last Updated on STN: 19980731
Entered Medline: 19980721

AB We have incorporated a bicyclic beta-turn mimetic (BTD; beta-turn dipeptide) into a zinc finger, creating a zinc finger with an artificial beta-turn. The designed peptide chelates zinc and has the same fold as the unmodified native zinc finger (finger 3 of the human YY1 protein). A combination of 1H NMR and structure calculations reveals that, in solution, this zinc finger has a fold similar to the known wild-type

crystal structure and to other zinc fingers containing the consensus sequence X3-Cys-X4-Cys-X12-His-X3-His-X. The peptide was designed with BTD between the chelating **cysteine** residues, with BTD forming a type II' beta-turn linking the two strands of a distorted anti-parallel beta-sheet. The C-terminal portion of the peptide forms a helix with zinc co-ordinating histidine residues on successive turns of the helix. This work represents a step towards developing methods by which parts of a target protein may be **replaced** by peptide mimetics.

L13 ANSWER 24 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1998256304 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9593720
TITLE: Alterations in the GAL4 DNA-binding domain can affect transcriptional activation independent of DNA binding.
AUTHOR: Corton J C; Moreno E; Johnston S A
CORPORATE SOURCE: Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina 27709-2137, USA.. corton@ciit.org
SOURCE: Journal of biological chemistry, (1998 May 29) 273 (22) 13776-80.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980713
Last Updated on STN: 20000303
Entered Medline: 19980701

AB The GAL4 protein belongs to a large class of fungal transcriptional activator proteins encoding within their DNA-binding domains (DBD) six **cysteines** that coordinate two atoms of zinc (the Zn2Cys6 domain). In an effort to characterize the interactions between the Zn2Cys6 class transcriptional activator proteins and their DNA-binding sites, we have **replaced** in the full-length GAL4 protein small regions of the Zn2Cys6 domain with the analogous regions of another Zn2Cys6 protein called PPR1 an activator of pyrimidine biosynthetic genes. Alterations between the first and third **cysteines** abolished binding to GAL4 (upstream activation sequence of GAL (UASG)) or PPR1 (upstream acitvation sequence of UAS) DNA-binding sites and severely reduced transcriptional activation in yeast. In contrast, alterations between the third and fourth **cysteines** had only minor effects on binding to UASG but led to substantial decreases in activation in both yeast and a mammalian cell line. In the **crystal** structure of the GAL4 DBD-UASG complex (Marmorstein, R., Carey, M., Ptashne, M., and Harrison, S. C. (1992) Nature 356, 408-414), this region is facing away from the DNA, making it likely that there exists within the GAL4 DBD an accessible domain important in activation.

L13 ANSWER 25 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1998201676 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9540799
TITLE: **Crystal** structures of 5-fluoro-dUrd and its 2 and/or 4-thio analogues: models of **substituted** dUMP pyrimidine ring interacting with thymidylate synthase.
AUTHOR: Jarmula A; Anulewicz R; Les A; Cyranski M K; Adamowicz L; Bretner M; Felczak K; Kulikowski T; Krygowski T M; Rode W
CORPORATE SOURCE: Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa, Poland.
SOURCE: Biochimica et biophysica acta, (1998 Feb 17) 1382 (2) 277-86.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980514
Last Updated on STN: 19980514

Entered Medline: 19980504

AB In order to understand the influence on thymidylate synthase interactions with dUMP analogues of the pyrimidine ring 2- and/or 4-thio, and 5-fluoro substitutions, X-ray diffractions by **crystals** of 5-fluoro-dUrd and its 2- and 4-thio, and 2,4-dithio analogues were measured, the four structures solved and refined. The following conclusions were suggested by results of comparative analyses of structural parameters (bond lengths, valence angles), followed by theoretical considerations based on calculated resonance structure distributions and aromaticity indices of the uracil, thiouracil, fluorouracil and fluorothiouracil rings. The effect of 4-thio substitution of FdUMP, altering specificity of inactivation of thymidylate synthases from various sources, is probably due to weaker proton acceptor power of the 4-thio substituent and increasing acidity (enhanced proton-donor power) of the N(3)-H moiety, resulting in an impaired fitness into the network of hydrogen bonds in the enzyme active center cleft. 2,4-Dithio substitution results in (i) impaired pyrimidine ring recognition by the enzyme active center, due to the 4-thio substituent (ii) increased pyrimidine ring aromaticity in dUMP, leading to resistance of C(6) to nucleophilic attack by the enzyme active center **cysteine** and (iii) altered planarity of the pyrimidine ring and deflections, with respect to the ring plane, of substituents at C(2), C(4) and C(5). 5-Fluoro substitution apparently activates the pyrimidine ring towards the interaction with thymidylate synthase by producing local strain, which results in an increased reactivity as predicted by the Walsh-Bent rule.

L13 ANSWER 26 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1998190029 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9521774
TITLE: Mode of action of site-directed irreversible folate analogue inhibitors of thymidylate synthase.
AUTHOR: Lobo A P; Nair M G; Changchien L; Weichsel A; Montfort W R; Maley F
CORPORATE SOURCE: Wadsworth Center, New York State Department of Health, Albany, New York 12201, USA.
CONTRACT NUMBER: CA27101 (NCI)
CA44355 (NCI)
SOURCE: Biochemistry, (1998 Mar 31) 37 (13) 4535-42.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980430
Last Updated on STN: 20000303
Entered Medline: 19980423

AB 5,8-Dideazafolate analogues are tight binding but not irreversible inhibitors of thymidylate synthase (TS). However, when a chloroacetyl (ClAc) group is **substituted** at the N10-position of 2-desamino-2-methyl-5,8-dideazafolate (DMDDF), the resulting compound, ClAc-DMDDF, although still a reversible inhibitor (KI = 3.4 x 10(-3) M), gradually inactivates thyA-TS irreversibly at a rate of 0.37 min-1. The corresponding iodoacetyl derivative alkylated the enzyme somewhat slower (k3 = 0.15 min-1) than ClAc-DMDDF but was bound more tightly (KI = 1.4 x 10(-5) M), resulting in a second-order rate constant (k3/KI) of inactivation that was 100-fold greater than that of ClAc-DMDDF. A tryptic digest of the ClAc-DMDDF-inactivated enzyme yielded a peptide on HPLC, which revealed that **cysteine**-146, the residue at the active site that is intimately involved in the catalytic process, had reacted with ClAc-DMDDF to form a covalent bond. This derivative was confirmed indirectly by Edman analysis and more directly by mass spectrometry. Deoxyuridine 5'-monophosphate, a substrate in the catalytic reaction, protected against inactivation. Similar to previously described Lactobacillus casei TS inhibition studies with sulfhydryl reagents [Galivan, J., Noonan, J., and Maley, F. (1977) Arch. Biochem. Biophys. 184, 336-345], the kinetics of inhibition suggested that complete inhibition occurs on reaction of only one of the two active site **cysteines**, although sequence and amino acid analysis revealed that iodoacetate and ClAc-DMDDF had reacted with both active site **cysteines**. These studies demonstrate that a sulfhydryl reactive

compound that is directed to the folate binding site of TS may diffuse to the active site **cysteine**, and form a covalent bond with this residue. How this inhibition comes about is suggested in a stereoscopic view of the ligand when modeled to the known **crystal** structure of Escherichia coli TS.

L13 ANSWER 27 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1998139965 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9466928
TITLE: The effects of steric mutations on the structure of type III antifreeze protein and its interaction with ice.
AUTHOR: DeLuca C I; Davies P L; Ye Q; Jia Z
CORPORATE SOURCE: Department of Biochemistry, Queen's University, Kingston, Ontario, Canada.
SOURCE: Journal of molecular biology, (1998 Jan 23) 275 (3) 515-25. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-2MSI; PDB-3MSI; PDB-4MSI; PDB-5MSI; PDB-6MSI; PDB-7MSI
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980312
Last Updated on STN: 20000303
Entered Medline: 19980305

AB The interaction of proteins with ice is poorly understood and difficult to study, partly because ice is transitory and can present many binding surfaces, and partly because structures have been determined for only two ice-binding proteins. This paper focuses on one of these, a 66-residue antifreeze protein (AFP) from eel pout. The high resolution X-ray structure of this fish AFP demonstrated that the proposed ice-binding surface is remarkably flat for such a small protein. The residues on the planar surface thought to be involved in ice binding are restrained by hydrogen bonds or by tight packing of their side-chains. To probe the requirement for a flat binding surface, a conserved alanine in the center of the AFP planar surface was **substituted** with larger residues. Six alanine replacement mutants (Ala16 > Cys, Thr, Met, Arg, His and Tyr), designed to disrupt the planarity of the surface and sterically block binding to ice, were characterized by X-ray crystallography and compared with the wild-type AFP. In each case, the detail provided by these **crystal** structures has helped explain the effects of the mutation on antifreeze activity. The substitutions, Ala16 > His and Ala16 > Tyr, were large enough to shield Gln44, one of the putative ice-binding residues, contributing to their very low thermal hysteresis activity. In addition to sterically hindering the putative ice-binding site, the bulkier residues also caused shifts in the putative ice-binding residues owing to the tight packing of side-chains on the planar surface. This unexpected consequence of the mutations helps account for the severely reduced antifreeze activity. One explanation for residual antifreeze activity in some of the mutants lies in the possibility that AFPs have a role in shaping the site on the ice to which they bind. Thus, side-chain dislocations might be partially accommodated by ice that can freeze around them. It is evident that the disruption of the planarity, by introducing larger residues at the center of the proposed ice-binding site, is not the only factor responsible for the loss of antifreeze activity. There are multiple causes including positional change and steric blockage of some putative ice-binding residues.

L13 ANSWER 28 OF 82 MEDLINE on STN
ACCESSION NUMBER: 1998087549 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9425098
TITLE: Movement of a loop in domain 3 of aerolysin is required for channel formation.
AUTHOR: Rossjohn J; Raja S M; Nelson K L; Feil S C; van der Goot F G; Parker M W; Buckley J T
CORPORATE SOURCE: The Ian Potter Foundation Protein Crystallography Laboratory, St. Vincent's Institute of Medical Research, 41 Victoria Parade, Fitzroy, Victoria 3065, Australia.
SOURCE: Biochemistry, (1998 Jan 13) 37 (2) 741-6. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980226
Last Updated on STN: 20000303
Entered Medline: 19980213

AB Aerolysin is a channel-forming toxin that must oligomerize in order to become insertion-competent. Modeling based on the **crystal** structure of the proaerolysin dimer and electron microscopic images of the oligomer indicated that a loop in domain 3 must move away from the beta-sheet that forms the main body of the protein before oligomerization can proceed. In order to determine if movement actually occurs, strategically located amino acids in the loop and in the sheet were **replaced** with **cysteines** by site-directed mutagenesis. A double mutant was produced in which the new **cysteines**, at position 253 on the loop and position 300 in the sheet, were close enough together to allow formation of a disulfide bridge. The double mutant was unable to oligomerize, and it was completely inactive, showing not only that the bridge had formed but also that movement of the loop was essential for formation of the oligomer. The existence of the bridge was confirmed by X-ray crystallography. The reduced form of the protein and the single mutants T253C and A300C were as active as wild type, indicating that the amino acid replacements themselves had no functional consequences. Labeling studies using an environment-sensitive fluorescent sulfhydryl-reactive probe confirmed that the structure of the protein changes in the loop region as a consequence of proteolytic activation of proaerolysin, a step which also must precede oligomerization.

L13 ANSWER 29 OF 82 MEDLINE on STN
ACCESSION NUMBER: 97452575 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9305946
TITLE: Sulfhydryl chemistry detects three conformations of the lipid binding region of Escherichia coli pyruvate oxidase.
AUTHOR: Chang Y Y; Cronan J E Jr
CORPORATE SOURCE: Department of Microbiology, University of Illinois, Urbana, Illinois 61801, USA.. yychang@pop.life.uiuc.edu
CONTRACT NUMBER: GM26156 (NIGMS)
SOURCE: Biochemistry, (1997 Sep 30) 36 (39) 11564-73.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971023

AB Site-specific disulfide cross-linking experiments detected a conformational change within the C-terminal segment of Escherichia coli pyruvate oxidase (PoxB), a lipid-activated homotetrameric enzyme, upon substrate binding [Chang, Y.-Y., & Cronan, J. E., Jr. (1995) J. Biol. Chem. 270, 7896-7901]. The C-terminal lipid binding regions were cross-linked only in the presence of the substrate, pyruvate, and the thiamine pyrophosphate cofactor, indicating close proximity of a pair of C termini. We have now systematically **substituted** **cysteine** at 18 additional amino acid positions within the C-terminal region to obtain a panel of 21 proteins each having a single residue changed to **cysteine**. These proteins have been studied by disulfide cross-linking and by accessibility of the **cysteine** side chain to a variety of sulfhydryl agents. In the absence of pyruvate, the **cysteine** residues of the modified PoxB proteins failed to form disulfide bonds, generally failed to react with a large and rigid hydrophilic sulfhydryl reagent, 4-acetamido-4'-[(iodoacetyl)amino]stilbene-2,2'-disulfonic acid (IASD), and in some cases reacted weakly with a smaller more hydrophobic reagent, N-ethylmaleimide. Therefore, in this conformation, the C termini appear fixed in a rigid environment having limited exposure to solvent. In the presence of pyruvate, all of the C-terminal **cysteine** residues (except the two most distal from the C terminus) reacted with both sulfhydryl reagents and readily formed disulfide cross-linked species, indicating conversion to a structure

having a high degree of conformational freedom. In the presence of lipid activators, Triton X-100 or dipalmitoylphosphatidylglycerol, a subset of the **cysteine-substituted** proteins no longer reacted with the membrane-impermeable IASD reagent, indicating penetration of these protein segments into the lipid micelles. For most of the proteins, similar extents of disulfide formation were seen upon addition of an oxidizing agent in the presence or absence of lipid activators. An exception was PoxB D560C which was much more readily cross-linked in the presence of lipid. Moreover, a subset of PoxB proteins that cross-linked to lower extents in the presence of lipids was found. The behavior of these proteins provides strong support for the model in which two C termini associate to form the functional lipid binding domain. These data are discussed in terms of three distinct PoxB conformers and the known **crystal** structure of a highly related protein.

L13 ANSWER 30 OF 82 MEDLINE on STN
ACCESSION NUMBER: 97426526 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9276751
TITLE: Probing the structure of the diphtheria toxin channel. Reactivity in planar lipid bilayer membranes of **cysteine-substituted** mutant channels with methanethiosulfonate derivatives.
AUTHOR: Huynh P D; Cui C; Zhan H; Oh K J; Collier R J; Finkelstein A
CORPORATE SOURCE: Department of Physiology & Biophysics, Albert Einstein College of Medicine, Bronx, New York 10461, USA.
CONTRACT NUMBER: GM-29210 (NIGMS)
T32-GM07288 (NIGMS)
SOURCE: Journal of general physiology, (1997 Sep) 110 (3) 229-42.
Journal code: 2985110R. ISSN: 0022-1295.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971021
Last Updated on STN: 20000303
Entered Medline: 19971009

AB Previous work has established that the 61 amino acid stretch from residue 322 to 382 in the T-domain of diphtheria toxin forms channels indistinguishable in ion-conducting properties from those formed by the entire T-domain. In the **crystal** structure of the toxin's water-soluble form, the bulk of this stretch is an alpha-helical hairpin, designated TH8-9. The present study was directed at determining which residues in TH8-9 line the ion-conducting pathway of the channel; i.e., its lumen or entrances. To this end, we singly mutated 49 of TH8-9's 51 residues (328-376) to **cysteines**, formed channels with the mutant T-domain proteins in planar lipid bilayers, and then determined whether they reacted with small, charged, lipid-insoluble, sulfhydryl-specific methanethiosulfonate (MTS) derivatives added to the bathing solutions. The indication of a reaction, and that the residue lined the ion-conducting pathway, was a sudden change in single-channel conductance and/or flickering behavior. The results of this study were surprising in two respects. First, of the 49 **cysteine-substituted** residues in TH8-9 tested, 23 reacted with MTS derivatives in a most unusual pattern consisting of two segments: one extending from 329 to 341 (11 of 13 reacted), and the other from 347 to 359 (12 of 13 reacted); none of the residues outside of these two segments appeared to react. Second, in every **cysteine** mutant channel manifesting an MTS effect, only one transition in single-channel conductance (or flickering behavior) occurred, not the several expected for a multimeric channel. Our results are not consistent with an alpha-helical or beta-strand model for the channel, but instead suggest an open, flexible structure. Moreover, contrary to common sense, they indicate that the channel is not multimeric but is formed from only one TH8-9 unit of the T-domain.

L13 ANSWER 31 OF 82 MEDLINE on STN
ACCESSION NUMBER: 97410385 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9261072
TITLE: Triosephosphate isomerase from Plasmodium falciparum: the **crystal** structure provides insights into

antimalarial drug design.
AUTHOR: Velanker S S; Ray S S; Gokhale R S; Suma S; Balaram H; Balaram P; Murthy M R
CORPORATE SOURCE: Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India.
SOURCE: Structure (London, England), (1997 Jun 15) 5 (6) 751-61. Journal code: 9418985. ISSN: 0969-2126.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19971008
Last Updated on STN: 20000421
Entered Medline: 19970925

AB BACKGROUND: Malaria caused by the parasite Plasmodium falciparum is a major public health concern. The parasite lacks a functional tricarboxylic acid cycle, making glycolysis its sole energy source. Although parasite enzymes have been considered as potential antimalarial drug targets, little is known about their structural biology. Here we report the **crystal** structure of triosephosphate isomerase (TIM) from P. falciparum at 2.2 A resolution. RESULTS: The **crystal** structure of P. falciparum TIM (PfTIM), expressed in Escherichia coli, was determined by the molecular replacement method using the structure of trypanosomal TIM as the starting model. Comparison of the PfTIM structure with other TIM structures, particularly human TIM, revealed several differences. In most TIMs the residue at position 183 is a glutamate but in PfTIM it is a leucine. This leucine residue is completely exposed and together with the surrounding positively charged patch, may be responsible for binding TIM to the erythrocyte membrane. Another interesting feature is the occurrence of a **cysteine** residue at the dimer interface of PfTIM (Cys13), in contrast to human TIM where this residue is a methionine. Finally, residue 96 of human TIM (Ser96), which occurs near the active site, has been **replaced** by phenylalanine in PfTIM. CONCLUSIONS: Although the human and Plasmodium enzymes share 42% amino acid sequence identity, several key differences suggest that PfTIM may turn out to be a potential drug target. We have identified a region which may be responsible for binding PfTIM to cytoskeletal elements or the band 3 protein of erythrocytes; attachment to the erythrocyte membrane may subsequently lead to the extracellular exposure of parts of the protein. This feature may be important in view of a recent report that patients suffering from P. falciparum malaria mount an antibody response to TIM leading to prolonged hemolysis. A second approach to drug design may be provided by the mutation of the largely conserved residue (Ser96) to phenylalanine in PfTIM. This difference may be of importance in designing specific active-site inhibitors against the enzyme. Finally, specific inhibition of PfTIM subunit assembly might be possible by targeting Cys13 at the dimer interface. The **crystal** structure of PfTIM provides a framework for new therapeutic leads.

L13 ANSWER 32 OF 82 MEDLINE on STN
ACCESSION NUMBER: 97383137 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9235976
TITLE: Mechanistic role of an NS4A peptide cofactor with the truncated NS3 protease of hepatitis C virus: elucidation of the NS4A stimulatory effect via kinetic analysis and inhibitor mapping.
AUTHOR: Landro J A; Raybuck S A; Luong Y P; O'Malley E T; Harbeson S L; Morgenstern K A; Rao G; Livingston D J
CORPORATE SOURCE: Vertex Pharmaceuticals Incorporated, 130 Waverly Street, Cambridge, Massachusetts 02139-4242, USA.
SOURCE: Biochemistry, (1997 Aug 5) 36 (31) 9340-8. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970916
Last Updated on STN: 20000303
Entered Medline: 19970902

AB Infection by hepatitis C viruses (HCVs) is a serious medical problem with no broadly effective treatment available for the progression of chronic hepatitis. The catalytic activity of a viral serine protease located in the N-terminal one-third of nonstructural protein 3 (NS3) is required for polyprotein processing at four site-specific junctions. The three-dimensional **crystal** structure of the NS3-NS4A co-complex [Kim, J. L., Morgenstern, K. A., Lin, C., Fox, T., Dwyer, M. D., Landro, J. A., Chambers, S. P., Markland, W., Lepre, C. A., O'Malley, E. T., Harbeson, S. L., Rice, C. M., Murcko, M. A., Caron, P. R., & Thomson, J. A. (1996) Cell 87, 343-355] delineates a small hydrophobic region within the 54-residue NS4A protein that intercalates with and makes extensive contacts to the core of the protease. The current investigation addresses the mechanism of NS3 protease catalytic activation by NS4A utilizing a small synthetic NS4A peptide (residues 1678-1691 of the virus polyprotein sequence) and the recombinantly expressed protease domain of NS3. The addition of NS4A dramatically increased NS3 kcat and kcat/Km catalytic parameters when measured against small peptide substrates representing the different site-specific junctions of the polyprotein. The catalytic effect of natural and non-natural amino acid substitutions at the P1 position in a 5A/5B peptide substrate was investigated. NS3-NS4A demonstrated a marked catalytic preference for the **cysteine** residue commonly found in authentic substrates. The pH dependence of the NS3 hydrolysis reaction is not affected by the presence of NS4A. This result suggests that NS4A does not change the pKa values of the active site residues of NS3 protease. A steady state kinetic analysis was performed and indicated that the binding of NS4A and the peptide substrate occurs in an ordered fashion during the catalytic cycle, with NS4A binding first. Two distinct kinetic classes of peptidyl inhibitors based upon the 5A/5B cleavage site were identified. An NS4A-independent class is devoid of prime residues. A second class of inhibitors is NS4A-dependent and contains a natural or non-natural cyclic amino acid **substituted** for the commonly found P1' residue serine. These inhibitors display an up to 80-fold increase in affinity for NS3 protease in the presence of NS4A. Sequential truncation of prime and P residues from this inhibitor class demonstrated the fact that the P4' and P1' residues are crucial for potent inhibition. The selectivity of this NS4A effect is interpreted using a model of the 5A/5B decapeptide substrate bound to the active site of the NS3-NS4A structure.

L13 ANSWER 33 OF 82 MEDLINE on STN
ACCESSION NUMBER: 97303055 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9159486
TITLE: NMR 15N relaxation and structural studies reveal slow conformational exchange in barstar C40/82A.
AUTHOR: Wong K B; Fersht A R; Freund S M
CORPORATE SOURCE: MRC Unit for Protein Function and Design, Cambridge Centre for Protein Engineering, University Chemical Laboratory, UK.
SOURCE: Journal of molecular biology, (1997 May 2) 268 (2) 494-511. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199706
ENTRY DATE: Entered STN: 19970630
Last Updated on STN: 19970630
Entered Medline: 19970616

AB Barstar an 89-residue protein consisting of four helices and a three-stranded parallel beta-sheet, is the intracellular inhibitor of the endoribonuclease barnase. Barstar C40/82A, a mutant in which the two **cysteine** residues have been **replaced** by alanine, has been used as a pseudo wild-type in folding studies and in the **crystal** structure of the barnase:barstar C40/82A complex. We have determined a high resolution solution structure of barstar C40/82A. The structures of barstar C40/82A and the wild-type are superimposable. A comparison with the **crystal** structure of the barnase:barstar C40/82A complex revealed subtle differences in the regions involved in the binding of barstar to barnase. Side-chain rotations of residues Asn33, Asp35 and Asp39 and a movement of the binding loop (Pro27-Glu32) towards the binding site of barnase facilitate the formation of interface hydrogen

bonds and aromatic contacts in the complex. Extreme line broadening and missing signals in 1H-15N correlation spectra indicate substantial conformational exchange for a large subset of residues. 15N relaxation data at two magnetic field strengths, 11.74 T and 14.10 T, were used to estimate exchange contributions and to map the spectral density function at five frequencies: 0, 50, 60, 450 and 540 MHz. Based on these results, model-free calculations with the inclusion of estimated exchange contributions were used to derive order parameters and internal correlation times. The validity of this approach has been investigated with model-free calculations that incorporate longitudinal relaxation rates and heteronuclear 1H-15N NOE data only at 11.74 T and 14.10 T. The relaxation data suggest substantial conformational exchange in regions of barstar C40/82A, including the binding loop, the second and the third helices, and the second and the third strands. Amide proton exchange experiments suggest a stable hydrogen bond network for all helices and sheets except the third helix and the C-terminal of the second and the third strands. The combined results indicate a rigid body movement of the second helix and twisting motions of the beta-sheet of barstar, which might be important for the interaction with barnase.

L13 ANSWER 34 OF 82 MEDLINE on STN
ACCESSION NUMBER: 97225918 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9122160
TITLE: Identification of a functional water channel in cytochrome P450 enzymes.
AUTHOR: Oprea T I; Hummer G; Garcia A E
CORPORATE SOURCE: Theoretical Biology and Biophysics Group (T-10), Los Alamos National Laboratory, NM 87545, USA.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1997 Mar 18) 94 (6) 2133-8. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970506
Last Updated on STN: 19970506
Entered Medline: 19970424

AB Cytochrome P450 enzymes are monooxygenases that contain a functional heme b group linked to a conserved **cysteine** with a thiolate bond. In the native state, the central iron atom is hexacoordinated with a covalently bound water molecule. The exclusion of solvent molecules from the active site is essential for efficient enzymatic function. Upon substrate binding, water has to be displaced from the active site to prevent electron uncoupling that results in hydrogen peroxide or water. In contrast to typical hemoproteins, the protein surface is not directly accessible from the heme of cytochromes P450. We postulate a two-state model in which a conserved arginine, stabilizing the heme propionate in all known cytochrome P450 **crystal** structures, changes from the initial, stable side-chain conformation to another rotamer (metastable). In this new state, a functional water channel (aqueduct) is formed from the active site to a water cluster located on the thiolate side of the heme, close to the protein surface. This water cluster communicates with the surface in the closed state and is partly **replaced** by the flipping arginine side chain in the open state, allowing water molecules to exit to the surface or to reaccess the active site. This two-state model suggests the presence of an exit pathway for water between the active site and the protein surface.

L13 ANSWER 35 OF 82 MEDLINE on STN
ACCESSION NUMBER: 97199393 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9047369
TITLE: **Crystal** structures of modified apo-His117Gly and apo-His46Gly mutants of Pseudomonas aeruginosa azurin.
AUTHOR: Hammann C; van Pouderoyen G; Nar H; Gomis Ruth F X; Messerschmidt A; Huber R; den Blaauwen T; Canters G W
CORPORATE SOURCE: Max Planck Institut fur Biochemie, Abteilung fur Struktur Forschung, Martinsried bei Munchen, Germany.
SOURCE: Journal of molecular biology, (1997 Feb 21) 266 (2) 357-66. Journal code: 2985088R. ISSN: 0022-2836.

PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970422
Last Updated on STN: 19970422
Entered Medline: 19970404

AB The X-ray **crystal** structures of two metal ligand mutants of azurin from Pseudomonas aeruginosa have been solved. In both mutants (His117Gly and His46Gly azurin) one of the copper coordinating histidine residues is **replaced** by a glycine, creating an empty space in the coordination sphere of the copper ion. The **crystal** structure of His117Gly azurin at 2.4 A resolution showed that this mutant had undergone partial oxidation at the disulfide bridge between Cys3 and Cys26 and full oxidation at the copper ligand Cys112. There is no copper present in the crystallized form and the bulky group of the oxidized **cysteine** at position 112 causes large structural rearrangements in the protein structure, especially in the loops connecting the beta-sheets. In the structure of the wild-type holo-azurin from P. aeruginosa the hydrophobic patch is important for the packing of the azurin molecules into dimers which then arrange into tetramers. The completely different packing of the apo-His117Gly mutant can be explained by the disruption of the hydrophobic patch area by the mutation-induced main-chain conformational change of residues 112 to 115. The structure of apo-His46Gly azurin at 2.5 A resolution is the same as the wild-type structure except for the immediate environment at the site of the mutation. In the His46Gly structure water molecules are found at positions that in the wild-type structure are occupied by the imidazole ring of His46 and the copper ion. The imidazole ring of His117 is shifted by about 1 A towards the surface of the protein, similar to that observed for 50% of the molecules in the wild-type apo-azurin structure. This shift causes a slight rearrangement of the monomers within the tetramer such that one local dyad becomes a crystallographic dyad parallel to the c-axis. This leads to a change in the space group from P2(1)2(1)2(1) to P2(1)2(1)2.

L13 ANSWER 36 OF 82 MEDLINE on STN
ACCESSION NUMBER: 97184692 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9032078
TITLE: Structure of a human lysosomal sulfatase.
AUTHOR: Bond C S; Clements P R; Ashby S J; Collyer C A; Harrop S J; Hopwood J J; Guss J M
CORPORATE SOURCE: Department of Biochemistry, University of Sydney, NSW 2006 Australia.
SOURCE: Structure (London, England), (1997 Feb 15) 5 (2) 277-89. Journal code: 9418985. ISSN: 0969-2126.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199706
ENTRY DATE: Entered STN: 19970612
Last Updated on STN: 19970612
Entered Medline: 19970603

AB BACKGROUND:. Sulfatases catalyze the hydrolysis of sulfuric acid esters from a wide variety of substrates including glycosaminoglycans, glycolipids and steroids. There is sufficient common sequence similarity within the class of sulfatase enzymes to indicate that they have a common structure. Deficiencies of specific lysosomal sulfatases that are involved in the degradation of glycosamino-glycans lead to rare inherited clinical disorders termed mucopolysaccharidoses. In sufferers of multiple sulfatase deficiency, all sulfatases are inactive because an essential post-translational modification of a specific active-site **cysteine** residue to oxo-alanine does not occur. Studies of this disorder have contributed to location and characterization of the sulfatase active site. To understand the catalytic mechanism of sulfatases, and ultimately the determinants of their substrate specificities, we have determined the structure of N-acetylgalactosamine-4-sulfatase. RESULTS:. The **crystal** structure of the enzyme has been solved and refined at 2.5 resolution using data recorded at both 123K and 273K. The structure has

two domains, the larger of which belongs to the alpha/beta class of proteins and contains the active site. The enzyme active site in the **crystals** contains several hitherto undescribed features. The active-site **cysteine** residue, Cys91, is found as the sulfate derivative of the aldehyde species, oxo-alanine. The sulfate is bound to a previously undetected metal ion, which we have identified as calcium. The structure of a vanadate-inhibited form of the enzyme has also been solved, and this structure shows that vanadate has **replaced** sulfate in the active site and that the vanadate is covalently linked to the protein. Preliminary data is presented for **crystals** soaked in the monosaccharide N-acetylgalactosamine, the structure of which forms a product complex of the enzyme. CONCLUSIONS:. The structure of N-acetylgalactosamine-4-sulfatase reveals that residues conserved amongst the sulfatase family are involved in stabilizing the calcium ion and the sulfate ester in the active site. This suggests an archetypal fold for the family of sulfatases. A catalytic role is proposed for the post-translationally modified highly conserved **cysteine** residue. Despite a lack of any previously detectable sequence similarity to any protein of known structure, the large sulfatase domain that contains the active site closely resembles that of alkaline phosphatase: the calcium ion in sulfatase superposes on one of the zinc ions in alkaline phosphatase and the sulfate ester of Cys91 superposes on the phosphate ion found in the active site of alkaline phosphatase.

L13 ANSWER 37 OF 82 MEDLINE on STN
ACCESSION NUMBER: 97153342 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9000632
TITLE: **Crystal** structures of 8-Cl and 9-Cl TIBO complexed with wild-type HIV-1 RT and 8-Cl TIBO complexed with the Tyr181Cys HIV-1 RT drug-resistant mutant.
AUTHOR: Das K; Ding J; Hsiou Y; Clark A D Jr; Moereels H; Koymans L; Andries K; Pauwels R; Janssen P A; Boyer P L; Clark P; Smith R H Jr; Kroeger Smith M B; Michejda C J; Hughes S H; Arnold E
CORPORATE SOURCE: Center for Advanced Biotechnology and Medicine and Department of Chemistry, Rutgers University, Piscataway, NJ 08854-5638, USA.
CONTRACT NUMBER: AI 27690 (NIAID)
AI 36144 (NIAID)
SOURCE: Journal of molecular biology, (1996 Dec 20) 264 (5) 1085-100.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
OTHER SOURCE: PDB-1HNV; PDB-1TVR; PDB-1UWB
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970227
Last Updated on STN: 19970227
Entered Medline: 19970212

AB Human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) is an important target for chemotherapeutic agents used in the treatment of AIDS; the TIBO compounds are potent non-nucleoside inhibitors of HIV-1 RT (NNRTIs). **Crystal** structures of HIV-1 RT complexed with 8-Cl TIBO (R86183, IC50 = 4.6 nM) and 9-Cl TIBO (R82913, IC50 = 33 nM) have been determined at 3.0 A resolution. Mutant HIV-1 RT, containing Cys in place of Tyr at position 181 (Tyr181Cys), is highly resistant to many NNRTIs and HIV-1 variants containing this mutation have been selected in both cell culture and clinical trials. We also report the **crystal** structure of Tyr181Cys HIV-1 RT in complex with 8-Cl TIBO (IC50 = 130 nM) determined at 3.2 A resolution. Averaging of the electron density maps computed for different HIV-1 RT/NNRTI complexes and from diffraction datasets obtained using a synchrotron source from frozen (-165 degrees C) and cooled (-10 degrees C) **crystals** of the same complex was employed to improve the quality of electron density maps and to reduce model bias. The overall locations and conformations of the bound inhibitors in the complexes containing wild-type HIV-1 RT and the two TIBO inhibitors are very similar, as are the overall shapes and volumes of the non-nucleoside inhibitor-binding pocket (NNIBP). The major differences between the two wild-type HIV-1 RT/TIBO complexes occur in the vicinity of

the TIBO chlorine substituents and involve the polypeptide segments around the beta5-beta6 connecting loop (residues 95 to 105) and the beta13-beta14 hairpin (residues 235 and 236). In all known structures of HIV-1 RT/NNRTI complexes, including these two, the position of the beta12-beta13 hairpin or the "primer grip" is significantly displaced relative to the position in the structure of HIV-1 RT complexed with a double-stranded DNA and in unliganded HIV-1 RT structures. Since the primer grip helps to position the template-primer, this displacement suggests that binding of NNRTIs would affect the relative positions of the primer terminus and the polymerase active site. This could explain biochemical data showing that NNRTI binding to HIV-1 RT reduces efficiency of the chemical step of DNA polymerization, but does not prevent binding of either dNTPs or DNA. When the structure of the Tyr181Cys mutant HIV-1 RT in complex with 8-Cl TIBO is compared with the corresponding structure containing wild-type HIV-1 RT, the overall conformations of Tyr181Cys and wild-type HIV-1 RT and of the 8-Cl TIBO inhibitors are very similar. Some positional changes in the polypeptide backbone of the beta6-beta10-beta9 sheet containing residue 181 are observed when the Tyr181Cys and wild-type complexes are compared, particularlty near residue Val179 of beta9. In the p51 subunit, the Cys181 side-chain is oriented in a similar direction to the Tyr181 side-chain in the wild-type complex. However, the electron density corresponding to the sulfur of the Cys181 side-chain in the p66 subunit is very weak, indicating that the thiol group is disordered, presumably because there is no significant interaction with either 8-Cl TIBO or nearby amino acid residues. In the mutant complex, there are slight rearrangements of the side-chains of other amino acid residues in the NNIBP and of the flexible dimethylallyl group of 8-Cl TIBO; these conformational changes could potentially compensate for the interactions that were lost when the relatively large tyrosine at position 181 was **replaced** by a less bulky **cysteine** residue. In the corresponding wild-type complex, Tyr181 iin the p66 subunit has significant interactions with the bound inhibitor and the position of the Tyr181 side-chain is well defined in both subunits. Apparently the Tyr181 --> Cys mutation eliminates favorable contacts of the aromatic ring of the tyrosine and the bou

L13 ANSWER 38 OF 82 MEDLINE on STN
ACCESSION NUMBER: 97133441 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8978838
TITLE: Design, synthesis, and evaluation of nonpeptidic inhibitors of human rhinovirus 3C protease.
AUTHOR: Webber S E; Tikhe J; Worland S T; Fuhrman S A; Hendrickson T F; Matthews D A; Love R A; Patick A K; Meador J W; Ferre R A; Brown E L; DeLisle D M; Ford C E; Binford S L
CORPORATE SOURCE: Agouron Pharmaceuticals, Inc., San Diego, California 92121, USA.
SOURCE: Journal of medicinal chemistry, (1996 Dec 20) 39 (26) 5072-82.
Journal code: 9716531. ISSN: 0022-2623.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 20000303
Entered Medline: 19970124

AB The design, synthesis, and biological evaluation of reversible, nonpeptidic inhibitors of human rhinovirus (HRV) 3C protease (3CP) are reported. A novel series of 2,3-dioxindoles (isatins) were designed that utilized a combination of protein structure-based drug design, molecular modeling, and structure-activity relationship (SAR). The C-2 carbonyl of isatin was envisioned to react in the active site of HRV 3CP with the **cysteine** responsible for catalytic proteolysis, thus forming a stabilized transition state mimic. Molecular-modeling experiments using the apo **crystal** structure of human rhinovirus-serotype 14 (HRV-14) 3CP and a peptide substrate model allowed us to design recognition features into the P1 and P2 subsites, respectively, from the 5- and 1-positions of isatin. Attempts to optimize recognition properties in the P1 subsite using SAR at the 5-position were performed. In addition, a series of ab initio calculations were carried out on several

5-**substituted** isatins to investigate the stability of sulfide adducts at C-3. The inhibitors were prepared by general synthetic methods, starting with commercially available 5-**substituted** isatins in nearly every case. All compounds were tested for inhibition of purified HRV-14 3CP. Compounds 8, 14, and 19 were found to have excellent selectivity for HRV-14 3CP compared to other proteolytic enzymes, including chymotrypsin and cathepsin B. Selected compounds were assayed for antiviral activity against HRV-14-infected HI-HeLa cells. A 2.8 A cocrystal structure of derivative 19 covalently bound to human rhinovirus-serotype 2 (HRV-2) 3CP was solved and revealed that the isatin was situated in essentially the same conformation as modeled.

L13 ANSWER 39 OF 82 MEDLINE on STN
ACCESSION NUMBER: 97039697 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8885249
TITLE: Determination of residues important in hormone binding to the extracellular domain of the luteinizing hormone/chorionic gonadotropin receptor by site-directed mutagenesis and modeling.
AUTHOR: Bhowmick N; Huang J; Puett D; Isaacs N W; Lapthorn A J
CORPORATE SOURCE: Department of Biochemistry & Molecular Biology, University of Georgia, Athens 30602, USA.
CONTRACT NUMBER: DK-33973 (NIDDK)
SOURCE: Molecular endocrinology (Baltimore, Md.), (1996 Sep) 10 (9) 1147-59.
Journal code: 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970130

AB The LH/CG receptor (LH/CG-R) belongs to the family of glycoprotein hormone G protein-coupled receptors. The extracellular domain of LH/CG-R is associated with high ligand-binding affinity and contains leucine-rich repeats (LRRs). With the goal of identifying essential amino acid residues involved in ligand binding, we **replaced** several conserved ionizable residues in the rat LH/CG-R with ones of opposite charge. The expression of these mutants was assessed by binding studies and Western blots after COS-7 cells were transiently transfected with wild type and mutant receptor cDNAs. The charge inversion of each of Lys40, Lys104, Asp118, Glu132, and Asp135 with Asp or Lys resulted in no detectable human CG binding in intact or solubilized cells; as control, a Lys40-->Arg replacement yielded a mutant with characteristics of the wild type receptor. Western analysis showed that the Lys40-->Arg mutant expressed at a level comparable to that of wild type receptor and, like wild type, exhibited a predominant immunoreactive mature form of LH/CG-R. Each of the five charge inversion mutants expressed at a lower level than wild type as assessed by immunoreactivity, and the levels of the Lys40-->Asp and Glu132-->Lys mutants were particularly low. The ratio of the mature to immature form of the receptor was high, i.e. like that of wild type, for the Glu132-->Lys and Asp135-->Lys replacements; the three other charge inversion mutants exhibited less mature than immature forms of the receptor. To aid in interpreting these results, we developed a model incorporating residues 27-235 of the extracellular domain of the rat LH/CG-R based on the **crystal** structure of the porcine ribonuclease inhibitor. Sequence homology and alignment revealed nine LRRs, with flanking **cysteine** clusters as found in a number of LRR proteins. Our model suggested that the Lys replacements of Glu132 and Asp135, i.e. those mutants that formed mature receptors, would disrupt the regional negative charge of the receptor. We propose that these residues are either directly involved in hormone binding or indirectly by disruption of the charge of an important binding surface.

L13 ANSWER 40 OF 82 MEDLINE on STN
ACCESSION NUMBER: 96406832 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8810919
TITLE: Characterization of the metal-binding sites of the beta-lactamase from Bacteroides fragilis.

AUTHOR: Crowder M W; Wang Z; Franklin S L; Zovinka E P; Benkovic S J
CORPORATE SOURCE: Department of Chemistry, Pennsylvania State University, University Park 16802, USA.
CONTRACT NUMBER: GM 16049 (NIGMS)
GM 18061 (NIGMS)
SOURCE: Biochemistry, (1996 Sep 17) 35 (37) 12126-32.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961029

AB In an effort to better understand the structure and function of the metallo-beta-lactamase from Bacteroides fragilis, spectroscopic and metal-binding studies were performed on the native, metal-**substituted**, and mutant forms of the enzyme. Atomic absorption studies demonstrate that the native B. fragilis enzyme tightly binds 2 mol of Zn(II) and, along with mutagenesis studies, that the presence of both metal ions is required for full catalytic activity. EPR spectroscopy was used to confirm that the Co(II)-**substituted** beta-lactamase binds 2 mol of Co(II) per mole of enzyme, that the two Co(II)'s are highspin and probably uncoupled, with apparent g values of 6.5, 4.2, and 2.0, and that the coordination number of the Co(II) is 5 or 6. This number of ligands for the Co(II)-**substituted** enzyme is confirmed by UV-Vis spectra, which demonstrate the presence of very weak d-d transitions between 550 and 650 nm (epsilon approximately 30 M-1.cm-1) and an intense feature at 320 nm (epsilon approximately 1570 M-1.cm-1). The latter is assigned to a **cysteine** sulfur to Co(II) ligand-to-metal charge transfer band, and this assignment is confirmed by the disappearance of this band in the UV-Vis spectrum of a Co(II)-**substituted** C168S mutant. H NMR studies on the Co(II)-**substituted** enzyme suggest the presence of three histidine ligands bound to Co(II). Taken together, these studies support the sequence comparison study of Rasmussen et al., in which there is a catalytic metal-binding site with three histidines and one **cysteine** (C168). The remaining ligands are postulated to be water molecules involved in catalysis. Mutagenesis studies, in combination with activity assays and metal-binding studies, have been used to identify Asp61, Asp90, Asp152, and Asp183 as possible ligands to the second metal-binding site, with Asp90 and Asp152 having a pronounced effect on kcat. These results are discussed in light of the recent **crystal** structure of the metallo-beta-lactamase from B. cereus.

L13 ANSWER 41 OF 82 MEDLINE on STN
ACCESSION NUMBER: 96354872 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8769310
TITLE: **Crystal** structure of a caricain D158E mutant in complex with E-64.
AUTHOR: Katerelos N A; Taylor M A; Scott M; Goodenough P W; Pickersgill R W
CORPORATE SOURCE: Institute of Food Research, Reading Laboratory, UK.
SOURCE: FEBS letters, (1996 Aug 19) 392 (1) 35-9.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961015
Last Updated on STN: 20000303
Entered Medline: 19961003

AB The structure of the D158E mutant of caricain (previously known as papaya protease omega) in complex with E-64 has been determined at 2.0 A resolution (overall R factor 19.3%). The structure reveals that the **substituted** glutamate makes the same pattern of hydrogen bonds as the aspartate in native caricain. This was not anticipated since in the native structure there is insufficient room to accommodate the glutamate side chain. The glutamate is accommodated in the mutant by a local

expansion of the structure demonstrating that small structural changes are responsible for the change in activity.

L13 ANSWER 42 OF 82 MEDLINE on STN
ACCESSION NUMBER: 96346069 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8718865
TITLE: Cys102 and His398 are required for bleomycin-inactivating activity but not for hexamer formation of yeast bleomycin hydrolase.
AUTHOR: Pei Z; Sebti S M
CORPORATE SOURCE: Department of Pharmacology, School of Medicine, University of Pittsburgh, Pennsylvania 15261, USA.
CONTRACT NUMBER: CA-48905 (NCI)
SOURCE: Biochemistry, (1996 Aug 20) 35 (33) 10751-6.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19961015
Last Updated on STN: 20000303
Entered Medline: 19960930

AB The bleomycin-inactivating enzyme, bleomycin hydrolase, is believed to be involved in tumor resistance to the anticancer drug bleomycin. This homohexamer is an aminopeptidase that shows homology to **cysteine** proteinases around the **cysteine** and histidine active site. The role that these residues play in hydrolyzing bleomycin and in hexamer oligomerization of bleomycin hydrolase is not known. In this study, the yeast bleomycin hydrolase gene was expressed in Escherichia coli, and site-directed mutagenesis was employed to precisely investigate the roles of the conserved Cys102 and His398 residues in its structure and enzymatic activity. Three mutants were created, in which Cys102 was **replaced** by arginine or serine, and His398 was changed to glycine. The ability of bleomycin hydrolase to oligomerize was neither affected by the subtle **cysteine**/serine mutation nor affected by **cysteine**/arginine or histidine/glycine mutations. However, the ability of bleomycin hydrolase to hydrolyze and inactivate bleomycin was totally abolished in all three mutants, suggesting that the **cysteine** thiol and histidine imidazole are critical for hydrolyzing bleomycin. Furthermore, in contrast to predictions from the recently reported **crystal** structure of this enzyme, hexamer formation is not required for the enzymatic activity of bleomycin hydrolase. Thus, these results demonstrate that Cys102 and His398 are required for bleomycin hydrolase activity but not hexamer formation, and that both monomer and hexamer are active forms of bleomycin hydrolase.

L13 ANSWER 43 OF 82 MEDLINE on STN
ACCESSION NUMBER: 96224848 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8639589
TITLE: Domain closure in adenylate kinase.
AUTHOR: Sinev M A; Sineva E V; Ittah V; Haas E
CORPORATE SOURCE: Department of Life Sciences, Bar-Ilan University, Ramat Gan, Israel.
SOURCE: Biochemistry, (1996 May 21) 35 (20) 6425-37.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960726
Last Updated on STN: 19980206
Entered Medline: 19960715

AB The method of time-resolved dynamic nonradiative excitation energy transfer (ET) was used to analyze the proposed domain closure in adenylate kinase (AK). A highly active mutant of Escherichia coli AK, (C77S, V169W, A55C)-AK, was prepared, in which the solvent- accessible residues valine 169 and alanine 55 were **replaced** by tryptophan (the donor of excitation energy) and **cysteine**, respectively. The latter was subsequently labeled with either 5- or 4-acetamidosalicylic acid (the

acceptor). From the comparative analysis of AK **crystal** structures [Schulz, G.E., Muller, C.W., & Diederichs, K. (1990) J. Mol. Biol. 213, 627-630] (apo-AK,AK.AMP complex and AK.AP5A [P1,P5-di(adenosine-5') pentaphosphate] complex), "sequential formation" of the pseudoternary AK.AP5A complex is followed by two- step domain closure. The domain closure reduces interdomain distances in a two-step manner. Specifically, the distance between C alpha-atoms of the residues 169 and 55 (numbers correspond to those of E. coli AK) is decreased from 23.6 A in the apo-enzyme to 16.2 A upon the formation of the AK.AMP complex and to 12.3 A upon the further formation of the pseudoternary AK.AP5A complex. Time-resolved dynamic nonradiative excitation energy transfer was measured for the following ligand forms of the labeled derivative of the mutant enzyme: the apo-enzyme, the enzyme-MgATP complex, the enzyme.AMP complex, and the enzyme.AP5A "ternary" complex. The transfer efficiencies, which were determined in these experiments, were approximately 7.5%, 22%, 33%, and 65%, respectively. Global analyses of the time resolved ET experiments with the same ligand forms yielded intermolecular distance distributions with corresponding means of 31, 23, 19, and 12 A and full widths at half- maximum of 29, 24, 14, and 11 A. The data confirmed the proposed stepwise manner of the domain closure of the enzyme and revealed the presence of multiple conformations of E. coli AK in solution.

L13 ANSWER 44 OF 82 MEDLINE on STN
ACCESSION NUMBER: 96214945 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8617792
TITLE: In vivo and in vitro iron-**replaced** zinc finger generates free radicals and causes DNA damage.
AUTHOR: Conte D; Narindrasorasak S; Sarkar B
CORPORATE SOURCE: Department of Biochemistry Research, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada.
SOURCE: Journal of biological chemistry, (1996 Mar 1) 271 (9) 5125-30.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960620
Last Updated on STN: 19970203
Entered Medline: 19960611

AB The estrogen receptor (ER) is a ligand-activated transcription factor whose DNA-binding domain (ERDBD) has eight **cysteines**, which coordinate two zinc atoms, forming two zinc finger-like structures. We demonstrate the capability of iron to replace zinc in zinc finger (hereby referred to as iron finger) both in vivo (using Escherichia coli BL21 (DE3)) and in vitro. Iron has the ability to substitute for zinc in the ERDBD as demonstrated by mobility shift and methylation interference assays of iron finger, which show specific recognition of the estrogen response element. The DNA binding constants for both in vivo and in vitro iron-**replaced** zinc fingers were similar to that of the native finger. Atomic absorption analysis revealed a ratio of 2:1 iron atoms/mol of ERDBD protein, as found for zinc in the **crystal** structure of native ERDBD. More importantly, we demonstrate that iron finger in the presence of H2O2 and ascorbate generates highly reactive free radicals, causing a reproducible cleavage pattern to the proximate DNA, the estrogen response element. The deoxyribose method, used to detect free radical species generated, and the resultant cleaved DNA ends, caused by iron finger, suggest that the free radicals generated are hydroxyl radicals. Due to the close proximity of the zinc finger to DNA, we postulate that iron-**substituted** zinc finger may generate free radicals while bound to genetic regulatory response elements, leading to adverse consequences such as iron-induced toxicity and/or carcinogenesis.

L13 ANSWER 45 OF 82 MEDLINE on STN
ACCESSION NUMBER: 96196618 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8611496
TITLE: Partitioning roles of side chains in affinity, orientation, and catalysis with structures for mutant complexes: asparagine-229 in thymidylate synthase.

AUTHOR: Finer-Moore J S; Liu L; Schafmeister C E; Birdsall D L; Mau T; Santi D V; Stroud R M
CORPORATE SOURCE: Department of Biochemistry and Biophysics, University of California, San Francisco 94143-0448, USA.
CONTRACT NUMBER: CA-14394 (NCI)
CA-41323 (NCI)
SOURCE: Biochemistry, (1996 Apr 23) 35 (16) 5125-36.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1NJA; PDB-1NJB; PDB-1NJC; PDB-1NJD; PDB-1NJE
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 19960613
Last Updated on STN: 20000303
Entered Medline: 19960606

AB Thymidylate synthase (TS) methylates only dUMP, not dCMP. The **crystal** structure of TS.dCMP shows sCMP 4-NH2 excluded from the space between Asn-229 and His-199 by the hydrogen bonding and steric properties and Asn-229. Consequently, 6-C of dCMP is over 4 A from the active site sulfhydryl. The Asn-229 side chain is prevented from flipping 180 degrees to and orientation the could hydrogen bond to dCMP by a hydrogen bond network between conserved residues. Thus, the specific binding of dUMP by TS results from occlusion of competing substrates by steric and electronic effects of residues in the active site cavity. When Asn-229 is **replaced** by a **cysteine**, the Cys-229 S gamma rotates out of the active site, and the mutant enzyme binds both dCMP and dUMP tightly but does not methylate dCMP. Thus simply admitting dCMP into the dUMP binding site of TS is not sufficient for methylation of dCMP. Structures of nucleotide complexes of TS N229D provide a reasonable explanation for the preferential methylation of dCMP instead of dUMP by this mutant. In TS N229D.dCMP, Asp-229 forms hydrogen bonds to 3-N and 40NH2 of dCMP. Neither the Asp-229 carboxyl moiety nor ordered water appears to hydrogen bond to 4-O of dUMP. Hydrogen bonds to 4-O (or 4-NH2) have been proposed to stabilize reaction intermediates. If their absence in TS N229D.dUMP persists in the ternary complex, it could explain the 10(4)-fold decrease in kcat/Km for dUMP.

L13 ANSWER 46 OF 82 MEDLINE on STN
ACCESSION NUMBER: 96070912 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7499249
TITLE: Maturation of pre-apocytochrome f in vivo. A site-directed mutagenesis study in Chlamydomonas reinhardtii.
AUTHOR: Kuras R; Buschlen S; Wollman F A
CORPORATE SOURCE: Service de Photosynthese, URA/CNRS 1187, Institut de Biologie Physico-chimique, Paris, France.
SOURCE: Journal of biological chemistry, (1995 Nov 17) 270 (46) 27797-803.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960217
Last Updated on STN: 20000303
Entered Medline: 19960117

AB The biosynthesis of cytochrome f is a multistep process which requires processing of the precursor protein and covalent ligation of a c-heme upon membrane insertion of the protein. The **crystal** structure of a soluble form of cytochrome f has revealed that one axial ligand of the c-heme is provided by the alpha-amino group of Tyr1 generated upon cleavage of the signal sequence from the precursor protein (Martinez S. E., Huang D., Szczepaniak A., Cramer W.A., and Smith J. L. (1994) Structure 2, 95-105). We therefore investigated, by site-directed mutagenesis, the possible interplay between protein processing and heme attachment to cytochrome f in Chlamydomonas reinhardtii. These modifications were performed by chloroplast transformation using a petA gene encoding the full-length precursor protein and also a truncated version lacking the C-terminal membrane anchor. We first

substituted the two cysteinyl residues responsible for covalent ligation of the c-heme, by a valine and a leucine, and showed that heme binding is not a prerequisite for cytochrome f processing. In another series of experiments, we **replaced** the consensus cleavage site for the thylakoid processing peptidase, AQA, by an LQL sequence. The resulting transformants were nonphototrophic and displayed delayed processing of the precursor form of cytochrome f, but nonetheless both the precursor and processed forms showed heme binding and assembled in cytochrome b6f complexes. Thus, pre-apocytochrome f adopts a suitable conformation for the cysteinyl residues to be substrates of the heme lyase and pre-holocytochrome f folds in an assembly-competent conformation. In the last series of experiments, we compared the rates of synthesis and degradation of the various forms of cytochrome f in the four types of transformants under study: (i) the C terminus membrane anchor apparently down-regulates the rate of synthesis of cytochrome f and (ii) degradation of misfolded forms of cytochrome f occurs by a proteolytic system intimately associated with the thylakoid membranes.

L13 ANSWER 47 OF 82 MEDLINE on STN
ACCESSION NUMBER: 96060099 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8520494
TITLE: Crystallization and preliminary X-ray diffraction studies of the human adenovirus serotype 2 proteinase with peptide cofactor.
AUTHOR: Keefe L J; Ginell S L; Westbrook E M; Anderson C W
CORPORATE SOURCE: Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, Illinois 60439, USA.
SOURCE: Protein science : a publication of the Protein Society, (1995 Aug) 4 (8) 1658-60.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960219
Last Updated on STN: 20020420
Entered Medline: 19960125

AB Recombinant human adenovirus serotype 2 proteinase (both native and selenomethionine-**substituted**) has been crystallized in the presence of the serotype 12, 11-residue peptide cofactor. The **crystals** (space group P3(1)21 or P3(2)21, one molecule per asymmetric unit, a = b = 41.3 angstrum, c = 197.0 angstrum) grew in solutions containing 20-40% 2-methyl-2,4-pentanediol (MPD), 0.1-0.2 M sodium citrate, and 0.1 M sodium HEPES, pH 5.0-7.5. Diffraction data (84% complete to 2.2 angstrum resolution with Rmerge of 0.0335) have been measured from cryopreserved native enzyme **crystals** with the Argonne blue (1,024 x 1,024 pixel array) charge-coupled device detector at beamline X8C at the National Synchrotron Light Source (operated by Argonne National Laboratory's Structural Biology Center). Additionally, diffraction data from selenomethionine-**substituted** proteinase, 65% complete to 2.0 angstrum resolution with Rmerge values ranging 0.05-0.07, have been collected at three X-ray energies at and near the selenium absorption edge. We have determined three of the six selenium sites and are initiating a structure solution by the method of multiwavelength anomalous diffraction phasing.

L13 ANSWER 48 OF 82 MEDLINE on STN
ACCESSION NUMBER: 96059309 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7591484
TITLE: Theoretical conformational analysis of three vasopressin antagonists with a modified cyclohexyl ring in the first thioacid residue.
AUTHOR: Kazmierkiewicz R; Liwo A; Lammek B
CORPORATE SOURCE: Department of Chemistry, University of Gdansk, Poland.
SOURCE: International journal of peptide and protein research, (1995 May) 45 (5) 451-8.
Journal code: 0330420. ISSN: 0367-8377.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199511
ENTRY DATE: Entered STN: 19960124
Last Updated on STN: 19960124
Entered Medline: 19951127

AB Analogues of arginine vasopressin (AVP) with bulky thioacid residues in position 1 of the amino acid sequence are known to be effective antagonists of the pressor response. Some of the most effective ones are those that have the first **cysteine** residue **replaced** with beta,beta-cyclopentamethylene-beta'-mercaptopropionic acid (Cpp) and its derivatives, such as 4-mercapto-4-tetrahydropyraneacetic acid (OCA) and 4-mercapto-4-tetrahydrothiopyraneacetic acid (SCA). The SCA analogues are more potent and the OCA ones slightly less potent antagonists than the Cpp ones. In this study we carried out conformational calculations on [Cpp1]AVP, [OCA1]AVP and [SCA1]AVP, using the ECEPP/3 force field both with and without hydration (to simulate an aqueous and non-polar receptor environment, respectively). It was found that most of the low-energy conformations are common in geometry and relative energy for all three compounds studied. It can therefore be concluded that the modifications of the cyclohexyl ring in position 1 influence the binding to the receptor because of changing the lipophilicity of the first residue, rather than by changing the conformational space. This is further supported by the fact that the lowest-energy conformations in the absence of water have closely located the Phe3 side chain (which is critical for the interaction with vasopressin receptors) and the (modified) cyclohexyl ring. The lowest-energy conformations in the presence and absence of water had beta-turns at residues Phe3-Gln4 and Gln4-Asn5, and Gln4-Asn5, respectively. The conformation with the turn at Gln4-Asn5 was most similar to the **crystal** structure of the pressinoic acid (the cyclic moiety of vasopressin).

L13 ANSWER 49 OF 82 MEDLINE on STN
ACCESSION NUMBER: 95399384 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7669757
TITLE: The Arg7Lys mutant of heat-labile enterotoxin exhibits great flexibility of active site loop 47-56 of the A subunit.
AUTHOR: van den Akker F; Merritt E A; Pizza M; Domenighini M; Rappuoli R; Hol W G
CORPORATE SOURCE: Department of Biological Structure and Biochemistry, University of Washington, Seattle, USA.
CONTRACT NUMBER: AI34501 (NIAID)
SOURCE: Biochemistry, (1995 Sep 5) 34 (35) 10996-1004.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951026
Last Updated on STN: 19951026
Entered Medline: 19951013

AB The heat-labile enterotoxin from Escherichia coli (LT) is a member of the cholera toxin family. These and other members of the larger class of AB5 bacterial toxins act through catalyzing the ADP-ribosylation of various intracellular targets including Gs alpha. The A subunit is responsible for this covalent modification, while the B pentamer is involved in receptor recognition. We report here the **crystal** structure of an inactive single-site mutant of LT in which arginine 7 of the A subunit has been **replaced** by a lysine residue. The final model contains 103 residues for each of the five B subunits, 175 residues for the A1 subunit, and 41 residues for the A2 subunit. In this Arg7Lys structure the active site cleft within the A subunit is wider by approximately 1 A than is seen in the wild-type LT. Furthermore, a loop near the active site consisting of residues 47-56 is disordered in the Arg7Lys structure, even though the new lysine residue at position 7 assumes a position which virtually coincides with that of Arg7 in the wild-type structure. The displacement of residues 47-56 as seen in the mutant structure is proposed to be necessary for allowing NAD access to the active site of the wild-type LT. On the basis of the differences observed between the wild-type and Arg7Lys structures, we propose a model for a coordinated

sequence of conformational changes required for full activation of LT upon reduction of disulfide bridge 187-199 and cleavage of the peptide loop between the two **cysteines** in the A subunit.(ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 50 OF 82 MEDLINE on STN
ACCESSION NUMBER: 95395846 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7666420
TITLE: **Crystal** structure of desulforedoxin from Desulfovibrio gigas determined at 1.8 A resolution: a novel non-heme iron protein structure.
AUTHOR: Archer M; Huber R; Tavares P; Moura I; Moura J J; Carrondo M A; Sieker L C; LeGall J; Romao M J
CORPORATE SOURCE: Instituto de Tecnologia Quimica e Biologica, Oeiras, Portugal.
CONTRACT NUMBER: GM 41482 (NIGMS)
SOURCE: Journal of molecular biology, (1995 Sep 1) 251 (5) 690-702. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951020
Last Updated on STN: 20000303
Entered Medline: 19951006

AB The **crystal** structure of desulforedoxin from Desulfovibrio gigas, a new homo-dimeric (2 x 36 amino acids) non-heme iron protein, has been solved by the SIRAS method using the indium-**substituted** protein as the single derivative. The structure was refined to a crystallographic R-factor of 16.9% at 1.8 A resolution. Native desulforedoxin **crystals** were grown from either PEG 4K or lithium sulfate, with cell constants a = b = 42.18 A, c = 72.22 A (for **crystals** grown from PEG 4K), and they belong to space group P3(2)21. The indium-**substituted** protein crystallized isomorphously under the same conditions. The 2-fold symmetric dimer is firmly hydrogen bonded and folds as an incomplete beta-barrel with the two iron centers placed on opposite poles of the molecule. Each iron atom is coordinated to four cysteinyl residues in a distorted tetrahedral arrangement. Both iron atoms are 16 A apart but connected across the 2-fold axis by 14 covalent bonds along the polypeptide chain plus two hydrogen bonds. Desulforedoxin and rubredoxin share some structural features but show significant differences in terms of metal environment and water structure, which account for the known spectroscopic differences between rubredoxin and desulforedoxin.

L13 ANSWER 51 OF 82 MEDLINE on STN
ACCESSION NUMBER: 95284031 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7766608
TITLE: Crystallographic analyses of NADH peroxidase Cys42Ala and Cys42Ser mutants: active site structures, mechanistic implications, and an unusual environment of Arg 303.
AUTHOR: Mande S S; Parsonage D; Claiborne A; Hol W G
CORPORATE SOURCE: Department of Biological Structure, School of Medicine, University of Washington, Seattle 98195, USA.
CONTRACT NUMBER: GM-35394 (NIGMS)
SOURCE: Biochemistry, (1995 May 30) 34 (21) 6985-92. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950713
Last Updated on STN: 20000303
Entered Medline: 19950703

AB NADH peroxidase from Enterococcus faecalis is a tetrameric flavoenzyme of 201,400 Da which employs Cys 42 as a redox-active center cycling between sulfhydryl (Cys-SH) and sulfenic acid (Cys-SOH) states along the catalytic pathway. The role of the active site **cysteine** 42 in NADH peroxidase has been elucidated using biochemical and crystallographic

techniques. Here we describe the **crystal** structures of two active site **cysteine** mutants, Cys42Ala and Cys42Ser, which were determined to 2.0 A resolution and refined to crystallographic R values of 17.6 and 18.3%, respectively. The overall chain fold and the quaternary structure of the two mutants appear to be very similar to wild-type enzyme. Therefore, the substantially lower activity of the mutants is due to the absence of the Cys-SOH redox center. One of the oxygen atoms of the nonnative **cysteine** sulfonic acid in the wild-type structure is **replaced** by a water molecule in both mutant structures. Two other residues near the active site are His 10 and Arg 303. A detailed analysis of the environment of these residues in the mutant and wild-type peroxidase structures indicates that the imidazole ring of His 10 is uncharged. The interactions made by the guanidinium group of Arg 303 involve not only His 10 but also the carboxylate of Glu 14 and Tyr 60. Interestingly, the Nn1H function of Arg 303 is oriented perpendicular to the plane of the phenyl ring of Tyr 60 with a Nn1 to phenyl ring center distance of 3.8 A, suggesting a favorable electrostatic interaction between Arg 303 and Tyr 60.(ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 52 OF 82 MEDLINE on STN
ACCESSION NUMBER: 95238289 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7721706
TITLE: Carbon monoxide-induced activation of gene expression in Rhodospirillum rubrum requires the product of cooA, a member of the cyclic AMP receptor protein family of transcriptional regulators.
AUTHOR: Shelver D; Kerby R L; He Y; Roberts G P
CORPORATE SOURCE: Department of Bacteriology, University of Wisconsin-Madison 53706.
CONTRACT NUMBER: 5 T32 GM08349 (NIGMS)
SOURCE: Journal of bacteriology, (1995 Apr) 177 (8) 2157-63. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U20508
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950605
Last Updated on STN: 19990129
Entered Medline: 19950519

AB Induction of the CO-oxidizing system of the photosynthetic bacterium Rhodospirillum rubrum is regulated at the level of gene expression by the presence of CO. In this paper, we describe the identification of a gene that is required for CO-induced gene expression. An 11-kb deletion of the region adjacent to the previously characterized cooFSCTJ region resulted in a mutant unable to synthesize CO dehydrogenase in response to CO and unable to grow utilizing CO as an energy source. A 2.5-kb region that corresponded to a portion of the deleted region complemented this mutant for its CO regulation defect, restoring its ability to grow utilizing CO as an energy source. When the 2.5-kb region was sequenced, one open reading frame, designated cooA, predicted a product showing similarity to members of the cyclic AMP receptor protein (CRP) family of transcriptional regulators. The product, CooA, is 28% identical (51% similar) to CRP and 18% identical (45% similar) to FNR from Escherichia coli. The insertion of a drug resistance cassette into cooA resulted in a mutant that could not grow utilizing CO as an energy source. CooA contains a number of **cysteine** residues **substituted** at, or adjacent to, positions that correspond to residues that contact cyclic AMP in the **crystal** structure of CRP. A model based on this observation is proposed for the recognition of CO by Cooa. Adjacent to cooA are two genes, nadB and nadC, with predicted products similar to proteins in other bacteria that catalyze reactions in the de novo synthesis of NAD.(ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 53 OF 82 MEDLINE on STN
ACCESSION NUMBER: 95187712 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7881909
TITLE: Structure of the catalytic core of the family F xylanase from Pseudomonas fluorescens and identification of the xylopentaose-binding sites.

AUTHOR: Harris G W; Jenkins J A; Connerton I; Cummings N; Lo Leggio L; Scott M; Hazlewood G P; Laurie J I; Gilbert H J; Pickersgill R W
CORPORATE SOURCE: Protein Engineering Department, Institute of Food Research, Reading Laboratory, UK.
SOURCE: Structure (London, England), (1994 Nov 15) 2 (11) 1107-16. Journal code: 9418985. ISSN: 0969-2126.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-P07986; GENBANK-P10478; GENBANK-P26514; GENBANK-P33559
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950425
Last Updated on STN: 20020917
Entered Medline: 19950407

AB BACKGROUND: Sequence alignment suggests that xylanases evolved from two ancestral proteins and therefore can be grouped into two families, designated F and G. Family F enzymes show no sequence similarity with any known structure and their architecture is unknown. Studies of an inactive enzyme-substrate complex will help to elucidate the structural basis of binding and catalysis in the family F xylanases. RESULTS: We have therefore determined the **crystal** structure of the catalytic domain of a family F enzyme, Pseudomonas fluorescens subsp. cellulosa xylanase A, at 2.5 A resolution and a crystallographic R-factor of 0.20. The structure was solved using an engineered catalytic core in which the nucleophilic glutamate was **replaced** by a **cysteine**. As expected, this yielded both high-quality mercurial derivatives and an inactive enzyme which enabled the preparation of the inactive enzyme-substrate complex in the **crystal**. We show that family F xylanases are eight-fold alpha/beta-barrels (TIM barrels) with two active-site glutamates, one of which is the nucleophile and the other the acid-base. Xylopentaose binds to five subsites A-E with the cleaved bond between subsites D and E. Ca2+ binding, remote from the active-site glutamates, stabilizes the structure and may be involved in the binding of extended substrates. CONCLUSIONS: The architecture of P. fluorescens subsp. cellulosa has been determined crystallographically to be a commonly occurring enzyme fold, the eight-fold alpha/beta-barrel. Xylopentaose binds across the carboxy-terminal end of the alpha/beta-barrel in an active-site cleft which contains the two catalytic glutamates.

L13 ANSWER 54 OF 82 MEDLINE on STN
ACCESSION NUMBER: 95119027 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7819235
TITLE: Analysis of the kinetic and redox properties of NADH peroxidase C42S and C42A mutants lacking the **cysteine**-sulfenic acid redox center.
AUTHOR: Parsonage D; Claiborne A
CORPORATE SOURCE: Department of Biochemistry, Wake Forest University Medical Center, Winston-Salem, North Carolina 27157-1016.
CONTRACT NUMBER: GM-35394 (NIGMS)
SOURCE: Biochemistry, (1995 Jan 17) 34 (2) 435-41. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 19950223
Last Updated on STN: 20000303
Entered Medline: 19950213

AB The flavoprotein NADH peroxidase from Enterococcus faecalis 10C1 has been shown to contain, in addition to FAD, an unusual **cysteine** -sulfenic acid (Cys-SOH) redox center. The non-flavin center cycles between reduced (Cys-SH) and oxidized (Cys-SOH) states, and the 2.16 A **crystal** structure of the non-native **cysteine**-sulfonic acid (Cys-SO3H) form of the wild-type peroxidase supports the proposed catalytic role of Cys42. In this study, we have employed a site-directed mutagenesis approach in which Cys42 is **replaced** with Ser and Ala, neither side chain of which is capable of redox activity. Reductive

titrations of both C42S and C42A mutants lead directly to full FAD reduction with 1 equiv of either dithionite or NADH, consistent with elimination of the Cys-SOH center. Direct determinations of the redox potentials for the FAD/FADH2 couples yield values of -219 and -197 mV, respectively, for C42S and C42A peroxidases, indicating that the presence of Cys42-SH in the two-electron-reduced wild-type enzyme lowers the flavin potential by approximately 100 mV. Anaerobic stopped-flow analyses of the reduction of C42S and C42A peroxidases by NADH demonstrate that in both cases flavin reduction is rapid; these results are confirmed by enzyme-monitored, steady-state kinetic analyses which, in addition, give turnover numbers approximately 0.04% that of wild-type enzyme. These results are entirely consistent with the role proposed for Cys42 in the catalytic redox cycle of wild-type NADH peroxidase and indirectly support its function as a peroxidatic center in the homologous NADH oxidase.

L13 ANSWER 55 OF 82 MEDLINE on STN
ACCESSION NUMBER: 94233032 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8177878
TITLE: Rapid crystallization of T4 lysozyme by intermolecular disulfide cross-linking.
AUTHOR: Heinz D W; Matthews B W
CORPORATE SOURCE: Institute of Molecular Biology, Howard Hughes Medical Institute, Eugene, OR.
CONTRACT NUMBER: GM21967 (NIGMS)
SOURCE: Protein engineering, (1994 Mar) 7 (3) 301-7.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940620
Last Updated on STN: 19940620
Entered Medline: 19940609

AB In an attempt to facilitate crystallization, engineered **cysteines** were used to promote formation of a 'back-to-back' dimer that occurs in different **crystal** forms of wild-type and mutant T4 lysozymes. The designed double mutant, N68C/A93C, in which the surface residues Asn68 and Ala93 were **replaced** by **cysteines**, formed dimers in solution and crystallized isomorphously to wild-type, but at a much faster rate. Overall, the mutant structure remained very similar to wild-type despite the formation of two intermolecular disulfide bridges. The **crystals** of cross-linked dimers ahd thermal factors somewhat lower than wild-type, indicating reduced mobility, but did not diffract to noticeably higher resolution. Introduction of the same cross-links was also used to obtain **crystals** in a different space group of a T4 lysozyme mutant that could not be crystallized previously. The results suggest that the formation of the lysozyme dimer is a critical intermediate in the formation of more than one **crystal** form and that covalent cross-linking of the intermediate accelerates nucleation and facilitates **crystal** growth. The disulfide cross-links are located on the 'back' of the molecule and formation of the cross-linked dimer appears to leave the active sites completely unobstructed. Nevertheless, the cross-linked dimer is completely inactive. One explanation for this behavior is that the disulfide bridges prevent hinge-bending motion that may be required for catalysis. Another possibility is that the formation of the dimer increases the overall bulk of the enzyme and prevents its access to the susceptible glycosidic bonds within the cell wall substrate.

L13 ANSWER 56 OF 82 MEDLINE on STN
ACCESSION NUMBER: 94227027 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8172874
TITLE: Flexible loop that is novel catalytic machinery in a ligase. Atomic structure and function of the loopless glutathione synthetase.
AUTHOR: Kato H; Tanaka T; Yamaguchi H; Hara T; Nishioka T; Katsube Y; Oda J
CORPORATE SOURCE: Institute for Chemical Research, Kyoto University, Japan.
SOURCE: Biochemistry, (1994 May 3) 33 (17) 4995-9.
Journal code: 0370623. ISSN: 0006-2960.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940620
Last Updated on STN: 19980206
Entered Medline: 19940608

AB The catalytic mechanism of glutathione synthetase is proposed to proceed via phosphorylation of the dipeptide substrate to yield an acyl phosphate intermediate; this intermediate is subsequently attacked by glycine, followed by loss of inorganic phosphate, leading to glutathione formation. A flexible loop (Ile226-Gly242) in Escherichia coli B glutathione synthetase is proposed to stabilize the acyl phosphate intermediate by preventing its decomposition by hydrolysis with water [Tanaka, T., Kato, H., Nishioka, T., & Oda, J. (1992) Biochemistry 31, 2259-2265; Tanaka, T., Yamaguchi, H., Kato, H., Nishioka, T., Katsube, Y., & Oda, J. (1993) Biochemistry 32, 12398-12404]. To investigate the function of the loop in the E. coli enzyme definitely, a loopless mutant in which the loop (Ile226-Arg241) was **replaced** with three residues of glycine was constructed. The **crystal** structure of the loopless mutant enzyme was essentially identical with that of the wild-type enzyme. Kinetic measurements showed that the replacement of the loop led to increases in the Km values, especially for the glycine, and a 930-fold decrease in the k0 value. Hence, the loopless mutant was 3 x 10(4) less active in terms of its specificity constant (k0/Km) for glycine than the wild-type enzyme. Moreover, the loopless mutant showed gamma-L-glutamyl-L-**cysteine**-dependent ATP hydrolase activity to almost the same extent as its glutathione synthetase activity. These studies support the fact that the loop enhances the recognition of glycine as well as stabilizes the acyl phosphate intermediate so that the intermediate rapidly reacts with glycine.

L13 ANSWER 57 OF 82 MEDLINE on STN
ACCESSION NUMBER: 94076360 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8254682
TITLE: Hepatitis A virus 3C proteinase: some properties, crystallization and preliminary crystallographic characterization.
AUTHOR: Chernaia M M; Malcolm B A; Allaire M; James M N
CORPORATE SOURCE: Department of Biochemistry, University of Alberta, Edmonton, Canada.
SOURCE: Journal of molecular biology, (1993 Dec 5) 234 (3) 890-3. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940203
Last Updated on STN: 20000303
Entered Medline: 19940112

AB Several isoforms of the wild-type and three mutant hepatitis A virus (HAV) 3C proteinases have been isolated and characterized. The active site **cysteine** residue (residue 172) was found to be responsible for the formation of some of these isoforms. The double mutant C24S/C172A of the HAV 3C proteinase, in which both **cysteine** residues have been **replaced** by site-directed mutagenesis, was crystallized. The **crystals** belong to the hexagonal space group P6(1)22 (or its enantiomorph, P6(5)22) with unit cell dimensions a = b = 65.2 A, c = 246.1 A and diffract X-rays to 2.3 A resolution.

L13 ANSWER 58 OF 82 MEDLINE on STN
ACCESSION NUMBER: 94073088 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8251947
TITLE: Identification of **cysteine** ligands in metalloproteins using optical and NMR spectroscopy: cadmium-**substituted** rubredoxin as a model [Cd(CysS)4]2- center.
AUTHOR: Henehan C J; Pountney D L; Zerbe O; Vasak M
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.

SOURCE: Protein science : a publication of the Protein Society, (1993 Oct) 2 (10) 1756-64.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940203
Last Updated on STN: 20000303
Entered Medline: 19940113

AB Optical and NMR methods are presented for the identification of **cysteine** ligands in Cd-**substituted** metalloproteins, in particular those containing zinc-fingerlike motifs, using Cd-**substituted** Desulfovibrio gigas rubredoxin (Cd-Rd) as a model [Cd(CysS)4]2- complex. The 113Cd NMR spectrum of Cd-Rd contains a single 113Cd resonance with a chemical shift position (723.6 ppm) consistent with tetrathiolate metal coordination. The proton chemical shifts of the four **cysteine** ligands were obtained from one-dimensional heteronuclear (1H-113Cd) multiple quantum coherence (HMQC) and total coherence spectroscopy (TOCSY)-relayed HMQC experiments. In addition, sequential assignments were made for two short **cysteine**-containing stretches of the polypeptide chain using a combination of homonuclear proton correlated spectroscopy, TOCSY, and nuclear Overhauser effect spectroscopy experiments, enabling sequence-specific heteronuclear 3J(1H beta-113Cd) coupling constants for each **cysteine** to be determined. The magnitude of these couplings (0-38 Hz) follows a Karplus-like dependence with respect to the H beta-C beta-S gamma-Cd dihedral angles, inferred from the **crystal** structure of the native protein. The difference absorption envelope (Cd-Rd vs. apo-Rd) reveals three distinct transitions with Gaussian-resolved maxima located at 213, 229, and 245 nm, which are paralleled by dichroic features in the corresponding difference CD and magnetic CD spectra. Based on the optical electronegativity theory of Jorgensen, the lowest energy transition has been attributed to a CysS-Cd(II) charge-transfer excitation (epsilon 245, 26,000 M-1 cm-1) with a molar extinction coefficient per **cysteine** of 6,500 M-1 cm-1.(ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 59 OF 82 MEDLINE on STN
ACCESSION NUMBER: 94032212 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8218166
TITLE: Intestinal fatty acid binding protein: characterization of mutant proteins containing inserted **cysteine** residues.
AUTHOR: Jiang N; Frieden C
CORPORATE SOURCE: Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110.
CONTRACT NUMBER: DK13332 (NIDDK)
SOURCE: Biochemistry, (1993 Oct 19) 32 (41) 11015-21.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199311
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 20000303
Entered Medline: 19931126

AB Site-directed mutagenesis was used to introduce **cysteine** residues into the rat intestinal fatty acid binding protein, an almost all beta-sheet protein that in the wild-type contains neither **cysteine** nor proline residues. Six mutants (I23C, S53C, V60C, L72C, L89C, and A104C) with a single **cysteine** residue **substituted** for a hydrophobic residue were characterized by their stability toward denaturants at pH 7.2 and 9.6, by their fluorescent properties, and by their reactivity toward the sulfhydryl modifying reagents 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and 4,4'-dipyridyl disulfide (4-PDS). In terms of protein stability, the substitutions were reasonably conservative with only two (V60C and L89C) being somewhat less stable than the wild-type. The mutant proteins differed considerably, however, in

their reactivity toward the modifying reagents. One residue, Cys89, located in a hydrophobic core near a turn between two beta-strands, was unreactive, while two residues, Cys60 and Cys104, located in the middle of beta-strands in the cavity into which fatty acid binds, reacted only very slowly and were further protected by oleate. Cys53, located near a turn and partially buried, appeared to have an unusually low pK value. Two residues, Cys23 and Cys72, reacted more rapidly in the native protein than in the unfolded protein. Both residues are located near the portal for the fatty acid binding, and one, Cys72, was strongly protected from modification by the presence of oleate. Examination of the **crystal** structure indicates that Cys72 is not easily solvent-accessible. We conclude that this high reactivity for this residue may be a consequence of rapid conformational flexibility in this region of the structure.

L13 ANSWER 60 OF 82 MEDLINE on STN
ACCESSION NUMBER: 93284091 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1284804
TITLE: Comparison of solution structures of mutant bovine pancreatic trypsin inhibitor proteins using two-dimensional nuclear magnetic resonance.
AUTHOR: Hurle M R; Eads C D; Pearlman D A; Seibel G L; Thomason J; Kosen P A; Kollman P; Anderson S; Kuntz I D
CORPORATE SOURCE: Department of Pharmaceutical Chemistry, University of California, San Francisco 94143.
CONTRACT NUMBER: GM19267 (NIGMS)
GM29072 (NIGMS)
RR01695 (NCRR)
SOURCE: Protein science : a publication of the Protein Society, (1992 Jan) 1 (1) 91-106.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 20000303
Entered Medline: 19930713

AB Structural perturbations due to a series of mutations at the 30-51 disulfide bond of bovine pancreatic trypsin inhibitor have been explored using NMR. The mutants **replaced cysteines** at positions 30 and 51 by alanine at position 51 and alanine, threonine, or valine at position 30. Chemical shift changes occur in residues proximate to the site of mutation. NOE assignments were made using an automated procedure, NASIGN, which used information from the wild-type **crystal** structure. Intensity information was utilized by a distance geometry algorithm, VEMBED, to generate a series of structures for each protein. Statistical analyses of these structures indicated larger averaged structural perturbations than would be expected from crystallographic and other information. Constrained molecular dynamics refinement using AMBER at 900 K was useful in eliminating structural movements that were not a necessary consequence of the NMR data. In most cases, statistically significant movements are shown to be those greater than approximately 1 A. Such movements do not appear to occur between wild type and A30A51, a result confirmed by crystallography (Eigenbrot, C., Randal, M., & Kossiakoff, A.A., 1990, Protein Eng. 3, 591-598). Structural alterations in the T30A51 or V30A51 mutant proteins near the limits of detection occur in the beta-loop (residues 25-28) or C-terminal alpha-helix, respectively.

L13 ANSWER 61 OF 82 MEDLINE on STN
ACCESSION NUMBER: 93187997 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8383207
TITLE: X-ray analysis and spectroscopic characterization of M121Q azurin. A copper site model for stellacyanin.
AUTHOR: Romero A; Hoitink C W; Nar H; Huber R; Messerschmidt A; Canters G W
CORPORATE SOURCE: Max-Planck Institut fur Biochemie, Abteilung Strukurforschung, Martinsried bei Munchen, Germany.
SOURCE: Journal of molecular biology, (1993 Feb 20) 229 (4)

1007-21.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 19930416
Last Updated on STN: 19970203
Entered Medline: 19930406

AB The dependence of the properties of the azurin blue copper site on the nature of the axial ligand at position 121 was tested by site-directed mutagenesis. This residue was **substituted** for a glutamine, the purported fourth copper ligand in the related protein stellacyanin. M121Q azurin was isolated and purified from Escherichia coli and characterized by spectroscopic methods. The mutant copper site has the ultra-violet-vis and electron paramagnetic resonance (EPR) characteristics of a type I site, but the spectroscopic details differ significantly from wild-type (wt) azurin. The X and S-band EPR spectra of M121Q azurin can be well stimulated with the parameters for stellacyanin, indicating that the copper sites of both proteins in the oxidized state are similar. The midpoint potential of M121Q is 263 mV, 25 mV lower than for wt azurin. The reactivity of the mutant was probed by measuring the electron self exchange rate by nuclear magnetic resonance spectroscopy. The rate was 8 x 10(3) mol-1 s-1, almost two orders of magnitude lower than the value for wt azurin (5 x 10(5) mol-1 s-1). Detailed structural information on the M121Q Cu(II)-site was obtained by X-ray analysis of M121Q azurin **crystals** at 1.9 A resolution. The histidine and **cysteine** copper ligand distances and angles in the equatorial plane around the copper are very similar to the wt protein. Gln121 is co-ordinated in a monodentate fashion via its side-chain oxygen atom at a distance of 2.26 A. The distance between copper and the carbonyl group of Gly45 is increased from 3.13 A (wt) to 3.37 A resulting in a distorted tetrahedral N2SO copper co-ordination. The possible significance of these results for the structure of the copper site of stellacyanin, the only small blue copper protein lacking a methionine ligand, is discussed. Conformational changes with respect to the wt azurin are seen in some of the connecting loops between secondary structure elements, in the mutation site and in the beta-strand 2a. The side-chains involved in the hydrophobic patch surrounding His117 are subject to large changes in their conformations. In contrast to wt azurin, the copper site in M121Q azurin undergoes significant structural changes on reduction.(ABSTRACT TRUNCATED AT 400 WORDS)

L13 ANSWER 62 OF 82 MEDLINE on STN
ACCESSION NUMBER: 93160188 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8431430
TITLE: Engineering the zinc binding site of human carbonic anhydrase II: structure of the His-94-->Cys apoenzyme in a new crystalline form.
AUTHOR: Alexander R S; Kiefer L L; Fierke C A; Christianson D W
CORPORATE SOURCE: Department of Chemistry, University of Pennsylvania, Philadelphia 19104-6323.
CONTRACT NUMBER: GM40602 (NIGMS)
SOURCE: Biochemistry, (1993 Feb 16) 32 (6) 1510-8.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930402
Last Updated on STN: 20000303
Entered Medline: 19930315

AB The structure of the His-94-->Cys variant of human carbonic anhydrase II (CAII) has been determined by X-ray crystallographic methods to a resolution of 2.3 A with a final crystallographic R factor of 0.155. This variant of CAII crystallizes in orthorhombic space group P2(1)2(1)2(1) which is the first example of a new **crystal** form for this important zinc hydrase (the wild-type enzyme crystallizes in monoclinic space group P21 under similar crystallization conditions). Although the

overall structure of the enzyme in the orthorhombic **crystal** form is similar to that of the wild-type protein in the monoclinic **crystal** form, the rms deviation of C alpha atoms between the two structures is 0.5 A. Larger structural deviations occur in regions of the protein molecule involved in **crystal** lattice contacts, and significant structural changes are found in the polypeptide strand containing Cys-94. Surprisingly, no electron density corresponding to a zinc ion is found in the active site of crystalline His-94-->Cys CAII, even though the stoichiometry of zinc binding to this variant in solution is confirmed by atomic absorption spectroscopy. However, the KD for zinc dissociation from the variant is increased 10(4)-fold compared with wild-type enzyme; furthermore, under the crystallization conditions of high ionic strength (1.75-2.5 M ammonium sulfate), the observed KD is increased further, which leads to zinc dissociation. Spectroscopic analysis of Co(2+)-**substituted** His-94-->Cys CAII indicates that the metal binds in a tetrahedral geometry with a new thiolate bond.(ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 63 OF 82 MEDLINE on STN
ACCESSION NUMBER: 93085739 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1453466
TITLE: Structure of oxidized bacteriophage T4 glutaredoxin (thioredoxin). Refinement of native and mutant proteins.
AUTHOR: Eklund H; Ingelman M; Soderberg B O; Uhlin T; Nordlund P; Nikkola M; Sonnerstam U; Joelson T; Petratos K
CORPORATE SOURCE: Department of Molecular Biology, Swedish University of Agricultural Sciences, Uppsala.
SOURCE: Journal of molecular biology, (1992 Nov 20) 228 (2) 596-618.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930129
Last Updated on STN: 19930129
Entered Medline: 19921231

AB The structure of wild-type bacteriophage T4 glutaredoxin (earlier called thioredoxin) in its oxidized form has been refined in a monoclinic **crystal** form at 2.0 A resolution to a crystallographic R-factor of 0.209. A mutant T4 glutaredoxin gives orthorhombic **crystals** of better quality. The structure of this mutant has been solved by molecular replacement methods and refined at 1.45 A to an R-value of 0.175. In this mutant glutaredoxin, the active site residues Val15 and Tyr16 have been **substituted** by Gly and Pro, respectively, to mimic that of Escherichia coli thioredoxin. The main-chain conformation of the wild-type protein is similar in the two independently determined molecules in the asymmetric unit of the monoclinic **crystals**. On the other hand, side-chain conformations differ considerably between the two molecules due to heterologous packing interactions in the **crystals** . The structure of the mutant protein is very similar to the wild-type protein, except at mutated positions and at parts involved in **crystal** contacts. The active site disulfide bridge between Cys14 and Cys17 is located at the first turn of helix alpha 1. The torsion angles of these residues are similar to those of Escherichia coli thioredoxin. The torsion angle around the S-S bond is smaller than that normally observed for disulfides: 58 degrees, 67 degrees and 67 degrees for wild-type glutaredoxin molecule A and B and mutant glutaredoxin, respectively. Each sulfur atom of the disulfide **cysteines** in T4 glutaredoxin forms a hydrogen bond to one main-chain nitrogen atom. The active site is shielded from solvent on one side by the beta-carbon atoms of the **cysteine** residues plus side-chains of residues 7, 9, 21 and 33. From the opposite side, there is a cleft where the sulfur atom of Cys14 is accessible and can be attacked by a nucleophilic thiolate ion in the initial step of the reduction reaction.

L13 ANSWER 64 OF 82 MEDLINE on STN
ACCESSION NUMBER: 91286292 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2061330
TITLE: The **crystal** structure of a mutant human lysozyme

C77/95A with increased secretion efficiency in yeast.
AUTHOR: Inaka K; Taniyama Y; Kikuchi M; Morikawa K; Matsushima M
CORPORATE SOURCE: Protein Engineering Research Institute, Osaka, Japan.
SOURCE: Journal of biological chemistry, (1991 Jul 5) 266 (19) 12599-603.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199108
ENTRY DATE: Entered STN: 19910825
Last Updated on STN: 20000303
Entered Medline: 19910807

AB The three-dimensional structure of a mutant human lysozyme, C77/95A, in which residues Cys77 and Cys95 were **replaced** by alanine, was determined at 1.8-A resolution by x-ray crystallography. The properties of this mutant protein have been well characterized with respect to its thermal stability and secretion efficiency in a yeast expression system. The overall three-dimensional structure of C77/95A was found to be essentially identical to that of the wild-type human lysozyme, although the coordinates were shifted by more than 0.5 A and the thermal factors of the main-chain atoms were increased in the vicinity of residue 77. The reduction in thermal stability of this mutant has been previously explained by an increase in entropy of the unfolded state. In addition, a packing defect (cavity) produced by the removal of the disulfide bond was detected in the three-dimensional structure of C77/95A. This cavity can also be a reason why the stability of the protein is reduced because the free energy of the folded state could be expected to increase. The increased secretion efficiency cannot be due mainly to the three-dimensional structure, but may possibly be related to some event in the pathway of protein secretion. One of the possibilities might involve molecular flexibilities in the secondary or tertiary structure for lack of one of the disulfide bonds.

L13 ANSWER 65 OF 82 MEDLINE on STN
ACCESSION NUMBER: 91269326 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1904942
TITLE: **Crystal** structure of rat trypsin-S195C at -150 degrees C. Analysis of low activity of recombinant and semisynthetic thiol proteases.
AUTHOR: Wilke M E; Higaki J N; Craik C S; Fletterick R J
CORPORATE SOURCE: Department of Biochemistry and Biophysics, University of California, San Francisco 94143-0448.
CONTRACT NUMBER: DK39304 (NIDDK)
SOURCE: Journal of molecular biology, (1991 Jun 5) 219 (3) 511-23.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199107
ENTRY DATE: Entered STN: 19910811
Last Updated on STN: 20000303
Entered Medline: 19910719

AB The X-ray **crystal** structure of trypsin-S195C, a rat anionic trypsin mutant in which the active site serine has been **replaced** by **cysteine**, was determined at -150 degrees C and room temperature to 1.6 A resolution, R = 15.4% and 1.8 A resolution, R = 15.0%, respectively. Cryo-crystallography was employed to improve the quality of the diffraction data and the resulting structure by eliminating radiation damage and decreasing atomic thermal motion. The average temperature factor decreased by 10 A2 relative to that of the room temperature structure. No radiation-induced decay of the data was detected. The side-chains of the catalytic **cysteine** and histidine of trypsin-S195C are found with 25% occupancy in secondary orientations rotated 104 degrees and 90 degrees out of the active site, respectively. These alterations, as well as more subtle changes in the active site may be caused by the oxidation of the catalytic sulfur to sulfenic acid. The position of the carbonyl carbon of the tetrahedral intermediate analog, p-amidinophenylpyruvic acid, modeled into

trypsin-S195C, is 1.1 A from the catalytic sulfur. The large size and altered approach of the catalytic sulfur to substrates could account for the observed low catalytic activity relative to wild-type trypsin. In addition to the benzamidine in the specificity pocket, two additional binding sites for benzamidine are characterized. One of these mediates an intermolecular contact that appears to maintain the **crystal** lattice.

L13 ANSWER 66 OF 82 MEDLINE on STN
ACCESSION NUMBER: 91093215 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1898729
TITLE: Bacillus subtilis alkaline phosphatases III and IV. Cloning, sequencing, and comparisons of deduced amino acid sequence with Escherichia coli alkaline phosphatase three-dimensional structure.
AUTHOR: Hulett F M; Kim E E; Bookstein C; Kapp N V; Edwards C W; Wyckoff H W
CORPORATE SOURCE: Department of Biological Sciences, University of Illinois, Chicago 60680.
SOURCE: Journal of biological chemistry, (1991 Jan 15) 266 (2) 1077-84.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M33634; GENBANK-M37165; GENBANK-M63255; GENBANK-M63505; GENBANK-M64332; GENBANK-M84122; GENBANK-M84123; GENBANK-M84124; GENBANK-M84125; GENBANK-M84128; GENBANK-S69830
ENTRY MONTH: 199102
ENTRY DATE: Entered STN: 19910322
Last Updated on STN: 19910322
Entered Medline: 19910212

AB Bacillus subtilis has an alkaline phosphatase multigene family. Two members of this gene family, phoAIII and phoAIV, were cloned, taking advantage of in vitro constructed strains containing a plasmid insertion within one or the other of the structural genes. The DNA sequences of the two genes showed approximately 64% identity at the DNA level and 63% identity in the deduced primary amino acid sequences. The phoAIII and phoAIV genes code for predicted proteins of 47,149 and 45,935 Da, respectively. Comparison of the deduced primary amino acid sequence of the mature proteins with other sequenced alkaline phosphatases from Escherichia coli, yeast, and humans shows 25-30% identity. Based on the refined **crystal** structure of E. coli alkaline phosphatase, it appears that the active site and the core of the structure are retained in both Bacillus alkaline phosphatases. However, both proteins are truncated at the amino terminus compared with other mature alkaline phosphatases, three sizable surface loops of E. coli are deleted, and a minidomain is **replaced** with a larger domain in the model. Neither Bacillus alkaline phosphatase sequenced contains any **cysteine** residues, an amino acid implicated in intrachain disulfide bond formation in other alkaline phosphatases.

L13 ANSWER 67 OF 82 MEDLINE on STN
ACCESSION NUMBER: 91017454 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1699222
TITLE: Structural effects induced by removal of a disulfide-bridge: the X-ray structure of the C30A/C51A mutant of basic pancreatic trypsin inhibitor at 1.6 A.
AUTHOR: Eigenbrot C; Randal M; Kossiakoff A A
CORPORATE SOURCE: Department of Biomolecular Chemistry, Genentech Inc., South San Francisco, CA 94080.
CONTRACT NUMBER: GM 33571-05 (NIGMS)
SOURCE: Protein engineering, (1990 Jul) 3 (7) 591-8.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199011

ENTRY DATE: Entered STN: 19910117
Last Updated on STN: 19960129
Entered Medline: 19901106

AB The X-ray structure of a variant of basic pancreatic trypsin inhibitor (BPTI) has been analyzed to determine the structural accommodation resulting from removal of a disulfide cross-link in a protein. The disulfide removed, Cys30-Cys51, has been implicated in both the folding pathway of the protein and its overall thermal stability. In the variant studied, C30A/C51A, the disulfide **cysteines** were **replaced** by less bulky alanines. The atomic displacements observed for C30A/C51A indicate a set of concerted shifts of two segments of chains, which together significantly diminish a packing defect at the site of the removed **cysteine** sulfur atoms. The observed structural changes are distributed asymmetrically around the sites of mutation, indicating that the adjacent beta-sheet is more resistant to the perturbation than the alpha-helix on the opposite side of the disulfide bond. The thermal parameters of groups involved in the structural accommodation are not significantly altered. A comparison of the X-ray structures reported for native BPTI determined in three different **crystal** forms indicates that the magnitude of its conformational variability exceeds that of the structural changes caused by the disulfide removal. This emphasizes the necessity of using isomorphous **crystal** systems to determine the relatively small effects due to mutation.

L13 ANSWER 68 OF 82 MEDLINE on STN
ACCESSION NUMBER: 90343783 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2116794
TITLE: Purification and characterization of Bacillus thuringiensis var. tenebrionis insecticidal proteins produced in E. coli.
AUTHOR: MacIntosh S C; McPherson S L; Perlak F J; Marrone P G; Fuchs R L
CORPORATE SOURCE: Monsanto Agricultural Company, Saint Louis, Missouri 63198.
SOURCE: Biochemical and biophysical research communications, (1990 Jul 31) 170 (2) 665-72.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901012
Last Updated on STN: 20000303
Entered Medline: 19900910

AB Native and single amino acid variants of the Bacillus thuringiensis var. tenebrionis insecticidal proteins were expressed in Escherichia coli, purified and examined for biological and biochemical properties. A novel, pH dependent, preferential precipitation method was implemented to purify Escherichia coli produced Bacillus thuringiensis var. tenebrionis proteins, which are active against Colorado potato beetle (Leptinotarsa decemlineata) larvae. **Cysteine** residues of the native Bacillus thuringiensis var. tenebrionis protein were **replaced** by serine residues by site-directed mutagenesis to investigate the biological and structural importance of the individual **cysteine** residues. Sulfhydryl determination of the native and amino acid variant Bacillus thuringiensis var. tenebrionis proteins revealed that the native protein contains no disulfide bonds. Modification of the carboxyl terminal **cysteine** residue (amino acid 540) caused complete inactivation of the protein. Native, truncated and single amino acid variants (other than at amino acid 540) exhibited insecticidal activities comparable to each other and to solubilized **crystals** from the original strain.

L13 ANSWER 69 OF 82 MEDLINE on STN
ACCESSION NUMBER: 90258028 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2342104
TITLE: X-ray **crystal** structure of a recombinant human myoglobin mutant at 2.8 A resolution.
AUTHOR: Hubbard S R; Hendrickson W A; Lambright D G; Boxer S G
CORPORATE SOURCE: Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032.

CONTRACT NUMBER: GM27738 (NIGMS)
SOURCE: Journal of molecular biology, (1990 May 20) 213 (2) 215-8.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199006
ENTRY DATE: Entered STN: 19900720
Last Updated on STN: 19900720
Entered Medline: 19900626

AB We have grown **crystals** in trigonal space group P3(2)21 of a mutant human myoglobin, aquomet form, in which lysine at position 45 has been **replaced** by arginine and **cysteine** at position 110 has been **replaced** by alanine. Suitable **crystals** of native recombinant human myoglobin have not been obtained. We have used the molecular replacement method to determine the X-ray **crystal** structure of the mutant at 2.8 A resolution. At the present stage of refinement, the crystallographic R-value for the model, with tightly restrained stereochemistry, is 0.158 for 5.0 to 2.8 A data. As expected, the overall structure is quite similar to the sperm whale myoglobin structure. Arginine 45 adopts a well-ordered conformation similar to that found in aquomet sperm whale myoglobin.

L13 ANSWER 70 OF 82 MEDLINE on STN
ACCESSION NUMBER: 90122788 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2611228
TITLE: **Crystal** structures of two engineered thiol trypsins.
AUTHOR: McGrath M E; Wilke M E; Higaki J N; Craik C S; Fletterick R J
CORPORATE SOURCE: Department of Biochemistry, University of California, San Francisco 94143-0448.
CONTRACT NUMBER: DK 39304 (NIDDK)
GM 11598 (NIGMS)
SOURCE: Biochemistry, (1989 Nov 28) 28 (24) 9264-70.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199003
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 20000303
Entered Medline: 19900314

AB We have determined the three-dimensional structures of engineered rat trypsins which mimic the active sites of two classes of **cysteine** proteases. The catalytic serine was **replaced** with **cysteine** (S195C) to test the ability of sulfur to function as a nucleophile in a serine protease environment. This variant mimics the **cysteine** trypsin class of thiol proteases. An additional mutation of the active site aspartate to an asparagine (D102N) created the catalytic triad of the papain-type **cysteine** proteases. Rat trypsins S195C and D102N,S195C were solved to 2.5 and 2.0 A, respectively. The refined structures were analyzed to determine the structural basis for the 10(6)-fold loss of activity of trypsin S195C and the 10(8)-fold loss of activity of trypsin D102N,S195C, relative to rat trypsin. The active site thiols were found in a reduced state in contrast to the oxidized thiols found in previous thiol protease structures. These are the first reported structures of serine proteases with the catalytic centers of sulfhydryl proteases. Structure analysis revealed only subtle global changes in enzyme conformation. The substrate binding pocket is unaltered, and active site amino acid 102 forms hydrogen bonds to H57 and S214 as well as to the backbone amides of A56 and H57. In trypsin S195C, D102 is a hydrogen-bond acceptor for H57 which allows the other imidazole nitrogen to function as a base during catalysis. In trypsin D102N,S195C, the asparagine at position 102 is a hydrogen-bond donor to H57 which places a proton on the imidazole nitrogen proximal to the nucleophile. This tautomer of H57 is unable to act as a base in catalysis.(ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 71 OF 82 MEDLINE on STN
ACCESSION NUMBER: 90105328 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2690937
TITLE: Putidaredoxin competitively inhibits cytochrome b5-cytochrome P-450cam association: a proposed molecular model for a cytochrome P-450cam electron-transfer complex.
AUTHOR: Stayton P S; Poulos T L; Sligar S G
CORPORATE SOURCE: Department of Biochemistry University of Illinois, Urbana 61801.
CONTRACT NUMBER: GM 31756 (NIGMS)
GM 33688 (NIGMS)
GM 33775 (NIGMS)
SOURCE: Biochemistry, (1989 Oct 3) 28 (20) 8201-5.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199002
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19970203
Entered Medline: 19900215

AB Cytochrome b5 has been genetically engineered to afford a fluorescent derivative capable of monitoring its association with cytochrome P-450cam from Pseudomonas putida [Stayton, P. S., Fisher, M. T., & Sligar, S. G. (1988) J. Biol. Chem. 263, 13544-13548]. In the mutant cytochrome b5, threonine is **replaced** by a **cysteine** at position 65 (T65C) and has been labeled with the environmentally sensitive fluorophore acrylodan. In this paper, the physiological P-450cam reductant putidaredoxin, an Fe2S2.Cys4 iron-sulfur protein, is shown to competitively inhibit the cytochrome b5 association, suggesting that cytochrome b5 and putidaredoxin bind to a similar site on the cytochrome P-450cam surface. Since the **crystal** structures for both cytochrome b5 and cytochrome P-450cam have been solved to high resolution, the complex has been computer modeled, and a good fit was found on the proximal surface of nearest approach to the P-450cam heme prosthetic group. The proposed model includes electrostatic contacts between conserved cytochrome b5 carboxylates Glu-44, Glu-48, Asp-60, and the exposed heme propionate with cytochrome P-450cam basic residues Lys-344, Arg-72, Arg-112, and Arg-364, respectively. Putidaredoxin has similarly been shown to contain a carboxylate-based binding surface, and the current results suggest that if the model is correct, then it also interacts at the proposed site, probably utilizing similar P-450cam electrostatic contacts.

L13 ANSWER 72 OF 82 MEDLINE on STN
ACCESSION NUMBER: 90074856 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2590771
TITLE: Relations between structure and nicotine-like activity: X-ray **crystal** structure analysis of (-)-cytisine and (-)-lobeline hydrochloride and a comparison with (-)-nicotine and other nicotine-like compounds.
AUTHOR: Barlow R B; Johnson O
CORPORATE SOURCE: Department of Pharmacology, Medical School, University Walk, Bristol.
SOURCE: British journal of pharmacology, (1989 Nov) 98 (3) 799-808.
Journal code: 7502536. ISSN: 0007-1188.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199001
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19970203
Entered Medline: 19900125

AB 1. Although (-)-cytisine is a rigid structure, it occurs in the **crystal** in two distinct but very similar conformations in which the pyridone ring is tilted relative to the charged nitrogen atom at much the same angle as the pyridine ring is in (-)-nicotine hydrogen iodide. The carbonyl group in the pyridone ring of (-)-cytisine, however, is on the side of the ring opposite to pyridine nitrogen in (-)-nicotine. 2.

The pKa of (-)-lobeline HCl at 25 degrees C is 8.6 (approx), indicating that (-)-lobeline is at least 90% in the protonated form at physiological pH (7.6). It is probably the phenyl 2-keto-ethyl part of (-)-lobeline, rather than the phenyl 2-hydroxy-ethyl part, which interacts with the receptor. 3. The combination within one molecule of a charged ('onium') nitrogen atom lying out of the plane of, and some distance (4.5-6.5 A) from, an aromatic ring is common to many compounds with nicotine-like activity (e.g. nicotine, cytisine, choline phenyl ether bromide, dimethyl-phenyl-piperazinium (DMPP) iodide, coryneine iodide and m-hydroxyphenylpropyl trimethyl ammonium iodide). In some molecules the aromatic ring can be **replaced** by an unsaturated group, such as carbonyl (e.g. acetylcholine) or double-bonds (e.g. anatoxin). 4. Activity at nicotinic receptors appears to involve interactions between the positively charged nitrogen atom and a negatively charged group, probably close to **cysteine** residues 192 and 193 in the receptor. It is suggested that rather than specific groups in the molecule also being involved, activity at nicotinic receptors depends on interactions between a flat part of the drug containing double-bonds, or systems of double bonds, and a planar area in the receptor, possibly tyrosine or phenylalanine residues.

L13 ANSWER 73 OF 82 MEDLINE on STN
ACCESSION NUMBER: 89346747 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2548279
TITLE: Conserved folding in retroviral proteases: **crystal** structure of a synthetic HIV-1 protease.
AUTHOR: Wlodawer A; Miller M; Jaskolski M; Sathyanarayana B K; Baldwin E; Weber I T; Selk L M; Clawson L; Schneider J; Kent S B
CORPORATE SOURCE: Crystallography Laboratory, NCI-Frederick Cancer Research Facility, MD 21701.
CONTRACT NUMBER: N01-CO-74101 (NCI)
SOURCE: Science, (1989 Aug 11) 245 (4918) 616-21.
Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 198909
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 20000303
Entered Medline: 19890912

AB The rational design of drugs that can inhibit the action of viral proteases depends on obtaining accurate structures of these enzymes. The **crystal** structure of chemically synthesized HIV-1 protease has been determined at 2.8 angstrom resolution (R factor of 0.184) with the use of a model based on the Rous sarcoma virus protease structure. In this enzymatically active protein, the **cysteines** were **replaced** by alpha-amino-n-butyric acid, a nongenetically coded amino acid. This structure, in which all 99 amino acids were located, differs in several important details from that reported previously by others. The interface between the identical subunits forming the active protease dimer is composed of four well-ordered beta strands from both the amino and carboxyl termini and residues 86 to 94 have a helical conformation. The observed arrangement of the dimer interface suggests possible designs for dimerization inhibitors.

L13 ANSWER 74 OF 82 MEDLINE on STN
ACCESSION NUMBER: 89302916 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2663067
TITLE: Escherichia coli thioredoxin folds into two compact forms of different stability to urea denaturation.
AUTHOR: Langsetmo K; Fuchs J; Woodward C
CORPORATE SOURCE: Department of Biochemistry, University of Minnesota, St. Paul 55108.
SOURCE: Biochemistry, (1989 Apr 18) 28 (8) 3211-20.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 198908
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19970203
Entered Medline: 19890817

AB The urea-induced denaturation of Escherichia coli thioredoxin and thioredoxin variants has been examined by electrophoresis on urea gradient slab gels by the method of Creighton [Creighton, T. (1986) Methods Enzymol. 131, 156-172]. Thioredoxin has only two **cysteine** residues, and these form a redox-active disulfide at the active site. Oxidized thioredoxin-S2 and reduced thioredoxin-(SH)2 each show two folded isomers with a large difference in stability to urea denaturation. The difference in stability is greater for the isomers of oxidized than for the isomers of reduced thioredoxin. At 2 degrees C, the urea concentrations at the denaturation midpoint are approximately 8 and 4.3 M for the oxidized isomers and 4.8 and 3.7 M for the reduced isomers. The difference between the gel patterns of samples applied in native versus denaturing buffer, and at 2 and 25 degrees C, is characteristic for the involvement of a cis-proline-trans-proline isomerization. The data very strongly suggest that the two folded forms of different stabilities correspond to the cis and trans isomers of the highly conserved Pro 76 peptide bond, which is cis in the **crystal** structure of oxidized thioredoxin. Urea gel experiments with the mutant thioredoxin P76A, with alanine **substituted** for proline at position 76, corroborate this interpretation. The electrophoretic banding pattern diagnostic for an involvement of proline isomerization in urea denaturation is not observed for oxidized P76A. In broad estimates of delta G degree for the native-denatured transition, the difference in delta G degree (no urea) between the putative cis and trans isomers of the Ile 75-Pro 76 peptide bond is approximately 3 kcal/mol for oxidized thioredoxin and approximately 1.5 kcal/mol for reduced thioredoxin. Since cis oxidized thioredoxin is much more stable than trans, folded oxidized thioredoxin is essentially all cis. In folded reduced thioredoxin, cis and trans interconvert slowly, on the minute time scale at 2 and 25 degrees C. In the absence of urea, the folded reduced thioredoxin is less than a few percent trans. Three additional mutants with additions or substitutions at the active site also show electrophoresis banding patterns consistent with a difference in stability between cis and trans isomers.

L13 ANSWER 75 OF 82 MEDLINE on STN
ACCESSION NUMBER: 89184395 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3507694
TITLE: Use of site-directed mutagenesis to obtain isomorphous heavy-atom derivatives for protein crystallography: **cysteine**-containing mutants of phage T4 lysozyme.
AUTHOR: Sun D P; Alber T; Bell J A; Weaver L H; Matthews B W
CORPORATE SOURCE: Institute of Molecular Biology, University of Oregon, Eugene 97403.
CONTRACT NUMBER: GM 10378 (NIGMS)
GM20066 (NIGMS)
GM21967 (NIGMS)
SOURCE: Protein engineering, (1987 Feb-Mar) 1 (2) 115-23.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198905
ENTRY DATE: Entered STN: 19900306
Last Updated on STN: 20000303
Entered Medline: 19890511

AB Five different **cysteine**-containing mutants of the lysozyme from bacteriophage T4 were used to explore the feasibility of using site-directed mutagenesis to generate isomorphous heavy-atom derivatives for protein crystallography. **Cysteines** 54 and 97, present in wild-type lysozyme, can be readily reacted with mercuric ion to produce an excellent isomorphous heavy-atom derivative. Mutants with an additional **cysteine** at position 86, 146, 153 or 157, or with Cys 97 **replaced** by Val, were engineered by site-directed mutagenesis. The mutant lysozyme Thr 157----Cys reacts with mercuric chloride to give an excellent new derivative although Cys 157 is only approximately 60% **substituted** with the heavy atom. The **cysteine** at

position 146 is largely buried but reacts readily with mercuric chloride. In this case the isomorphism is poor and the resultant derivative is of marginal quality. Cys 153 reacts rapidly with mercuric ion but the derivative **crystals** do not diffract. The mutant Pro 86----Cys does not yield a particularly good heavy-atom derivative. This is due in part to a loss of isomorphism associated with the mutation. In addition, Cys 86 shows very little reactivity towards mercurials even though it is fully exposed to solvent. The mutation Cys 97----Val was used to explore the possibility of creating an independent derivative by deleting a heavy-atom site already present in wild-type lysozyme. In all cases that were tested, the quality of the heavy-atom derivative was improved by using as an isomorphous pair mercury-**substituted** mutant versus non-**substituted** mutant rather than mercury-**substituted** mutant versus (non-**substituted**) wild-type lysozyme.(ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 76 OF 82 MEDLINE on STN
ACCESSION NUMBER: 88314996 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3137220
TITLE: The central helix of calmodulin functions as a flexible tether.
AUTHOR: Persechini A; Kretsinger R H
CORPORATE SOURCE: Department of Biology, University of Virginia, Charlottesville 22901.
SOURCE: Journal of biological chemistry, (1988 Sep 5) 263 (25) 12175-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Space Life Sciences
ENTRY MONTH: 198809
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 20000303
Entered Medline: 19880928

AB Using site-directed mutagenesis we have created an altered calmodulin in which Gln-3 and Thr-146 have both been **replaced** by **cysteines**. We have reacted this protein with the bifunctional reagent, bismaleimidohexane, forming an intramolecular cross-link between the two **cysteines**. In the **crystal** structure of native calmodulin alpha-carbons at positions 3 and 146 are 37 A apart. In the bismaleimidohexane cross-linked protein these atoms can be no more than 19 A apart, and model building studies indicate that there is probably a bend in the central helix of calmodulin. A second modified calmodulin was generated by cleaving the central helix of the cross-linked protein at Lys-77 with trypsin. In this molecule, the two lobes of calmodulin are joined solely by the bismaleimidohexane cross-link, which bridges Cys-3 and Cys-146. Vm and Kact values for activation of myosin light chain kinase activity by the cross-linked and cross-linked/trypsinized proteins are not significantly different from those for the control protein. This result indicates that one role for the central helix may be to serve as a flexible tether between the calmodulin lobes. This is consistent with a model calmodulin-enzyme complex in which the central helix is bent, and the two lobes exert a concerted effect. A detailed model of this type has been proposed for the calmodulin-myosin light chain kinase complex (Persechini, A. and Kretsinger, R.H. (1988) J. Cardiovasc. Pharmacol., in press).

L13 ANSWER 77 OF 82 MEDLINE on STN
ACCESSION NUMBER: 87308240 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3497924
TITLE: **Crystals** and a low resolution structure of interleukin-2.
AUTHOR: Brandhuber B J; Boone T; Kenney W C; McKay D B
CONTRACT NUMBER: AI-00631 (NIAID)
AI-19762 (NIAID)
SOURCE: Journal of biological chemistry, (1987 Sep 5) 262 (25) 12306-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198710
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19871009

AB Recombinant derived human interleukin-2 and an analog in which **cysteine** 125 has been **replaced** with alanine have been crystallized in a form suitable for x-ray diffraction. The **crystals** are triclinic, space group P1, with two protein molecules in the unit cell; unit cell parameters are a = 55.8 A, b = 40.1 A, c = 33.7 A, alpha = 90.0 degrees, beta = 109.3 degrees, gamma = 93.2 degrees. The interleukin-2 structure has been solved to 5.5 A resolution using heavy atom isomorphous replacement methods. The resultant low resolution model reveals a significant fraction of alpha helical secondary structure and outlines the overall tertiary structure of the molecule.

L13 ANSWER 78 OF 82 MEDLINE on STN
ACCESSION NUMBER: 87194753 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3032940
TITLE: **Crystal** structure of a cyclic AMP-independent mutant of catabolite gene activator protein.
AUTHOR: Weber I T; Gilliland G L; Harman J G; Peterkofsky A
SOURCE: Journal of biological chemistry, (1987 Apr 25) 262 (12) 5630-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198706
ENTRY DATE: Entered STN: 19900303
Last Updated on STN: 19900303
Entered Medline: 19870605

AB Escherichia coli NCR91 synthesizes a mutant form of catabolite gene activator protein (CAP) in which alanine 144 is **replaced** by threonine. This mutant, which also lacks adenylate cyclase activity, has a CAP phenotype; in the absence of cAMP it is able to express genes that normally require cAMP. CAP91 has been purified and crystallized with cAMP under the same conditions as used to crystallize the wild type CAP X cAMP complex. X-ray diffraction data were measured to 2.4-A resolution and the CAP91 structure was determined using initial model phases from the wild type structure. A difference Fourier map calculated between CAP91 and wild type showed the 2 alanine to threonine sequence changes in the dimer and also a change in orientation of **cysteine** 178 in one of the subunits. The CAP91 coordinates were refined by restrained least squares to an R factor of 0.186. Differences in the atomic positions of the wild type and mutant protein structures were analyzed by a vector averaging technique. There were small changes that included concerted motions in the small domains, in the hinge between the two domains and in an adjacent loop between beta-strands 4 and 5. The mutation at residue 144 apparently causes changes in the position of some protein atoms that are distal to the mutation site.

L13 ANSWER 79 OF 82 MEDLINE on STN
ACCESSION NUMBER: 86026237 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2932154
TITLE: X-ray analysis of structural changes induced by reduced nicotinamide adenine dinucleotide when bound to **cysteine**-46-carboxymethylated liver alcohol dehydrogenase.
AUTHOR: Cedergren-Zeppezauer E S; Andersson I; Ottonello S; Bignetti E
SOURCE: Biochemistry, (1985 Jul 16) 24 (15) 4000-10.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198512
ENTRY DATE: Entered STN: 19900321

Last Updated on STN: 20000303
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AB The structure of the complex between Cys-46-carboxymethylated horse liver alcohol dehydrogenase (CM-LADH) and reduced nicotinamide adenine dinucleotide (NADH) has been determined by X-ray analysis. The complex represents NADH binding to the orthorhombic, "open" conformation of the enzyme. Coenzyme binding here induces a local structural change in the peptide loop 293-297, but there is no domain rotation, as observed for the "closed" conformation of the protein. This local movement of a few residues in the loop is sufficient to trap the nicotinamide ring of NADH within the active-site area close to a productive binding position. The carboxymethyl group on the zinc ligand **cysteine**-46 is oriented between the pyrophosphate bridge of NADH and the guanidinium group of arginine-369 and can occupy this position because the coenzyme binding cleft remains open and unchanged upon coenzyme binding. The zinc coordination sphere is distorted, and the position of the metal atom is shifted 1 A compared to native unliganded LADH. The distance between the zinc ion and the sulfur of the alkylated **cysteine** residue is of the order of 3 A. Alkylation experiments were performed at 0.15 and 10 mM iodoacetate, and peptide maps were examined. Gentle treatment with reagent yields an enzyme product which is **substituted** at only one of the two zinc binding sites per subunit of LADH (Cys-46). This enzyme species maintains its structural integrity; it binds coenzyme which induces conformational changes resolved into two steps. Thus, in addition to the orthorhombic complex, a crystalline NADH complex in the closed conformation of CM-LADH was obtained. These **crystals** showed enzymic activity, and single **crystals** were analyzed with microspectrophotometric methods. Formation of the stable crystalline abortive complex between CM-LADH-NAD+ and 4-trans-(N,N-dimethylamino)cinnamaldehyde (DACA) could be observed upon addition of excess aldehyde to the closed complex of CM-LADH-NADH. The CM-LADH-NAD+-DACA complex is characterized by an intense absorption band with a lambda max at 456 nm which corresponds to a shift in the spectrum of free DACA of approximately 60 nm. At the higher concentration of iodoacetate, three of the **cysteine** ligands to the second zinc atom (Cys-100, -103, and -111) are alkylated in addition to Cys-46. This enzyme product rapidly denatures and cannot be crystallized under our conditions. This is an experimental indication that the intact noncatalytic zinc binding site contributes to the structural stability of the protein.

L13 ANSWER 80 OF 82 MEDLINE on STN
ACCESSION NUMBER: 84044756 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6356360
TITLE: Directed mutagenesis of dihydrofolate reductase.
AUTHOR: Villafranca J E; Howell E E; Voet D H; Strobel M S; Ogden R C; Abelson J N; Kraut J
CONTRACT NUMBER: CA17374 (NCI)
F32 GM09375 (NIGMS)
GM10928 (NIGMS)
SOURCE: Science, (1983 Nov 18) 222 (4625) 782-8.
Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198312
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19831217

AB Three mutations of the enzyme dihydrofolate reductase were constructed by oligonucleotide-directed mutagenesis of the cloned Escherichia coli gene. The mutations--at residue 27, aspartic acid **replaced** with asparagine; at residue 39, proline **replaced** with **cysteine**; and at residue 95, glycine **replaced** with alanine--were designed to answer questions about the relations between molecular structure and function that were raised by the x-ray **crystal** structures. Properties of the mutant proteins show that Asp-27 is important for catalysis and that perturbation of the local structure at a conserved cis peptide bond following Gly-95 abolishes activity. Substitution of **cysteine** for proline at residue 39

results in the appearance of new forms of the enzyme that correspond to various oxidation states of the **cysteine**. One of these forms probably represents a species cross-linked by an intrachain disulfide bridge between the **cysteine** at position 85 and the new **cysteine** at position 39.

L13 ANSWER 81 OF 82 MEDLINE on STN
ACCESSION NUMBER: 84042883 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6356167
TITLE: Structural studies of horse liver alcohol dehydrogenase: coenzyme, substrate and inhibitor binding.
AUTHOR: Eklund H
SOURCE: Pharmacology, biochemistry, and behavior, (1983) 18 Suppl 1 73-81.
Journal code: 0367050. ISSN: 0091-3057.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198312
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19980206
Entered Medline: 19831220

AB Alcohol dehydrogenase from horse liver has been thoroughly investigated with crystallographic methods. Four different **crystal** forms of the enzyme have been solved and refined. They show that the enzyme exists in two predominant forms. The open form is found in the absence of coenzyme and has two long deep clefts cutting the enzyme in three units. In the closed form of the enzyme these clefts are closed around the coenzyme and substrate/inhibitor. Although there are large conformational changes in the enzyme, they are mainly restricted to relative movements of the separate domains. The internal structure of these domains is virtually identical in the open and closed forms. The coenzyme is the main cause of the conformational change and binds with a large number of interactions to the enzyme. About 4% of the enzyme surface is covered by the bound coenzyme. The nicotinamide ring is not bound to the active site zinc atom, but puts one surface of the ring in contact with the zinc coordinated **cysteine** sulphur atoms. The oxygen atom of the substrate binds directly to the zinc atom with the rest of the substrate close to the nicotinamide of the coenzyme. Large substrates extend into a 15-20 A long hydrophobic channel which opens up towards the solution. The widely used inhibitor pyrazole binds as a bridge between the zinc atom and the nicotinamide ring. Pyrazoles **substituted** in the 4-position are generally strong inhibitors. This can be properly related to the organization of the substrate channel of the enzyme.

L13 ANSWER 82 OF 82 MEDLINE on STN
ACCESSION NUMBER: 83299959 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6351056
TITLE: **Crystal** structures of the active site in specifically metal-depleted and cobalt-**substituted** horse liver alcohol dehydrogenase derivatives.
AUTHOR: Schneider G; Eklund H; Cedergren-Zeppezauer E; Zeppezauer M
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1983 Sep) 80 (17) 5289-93.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198310
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19831008

AB Two derivatives of horse liver alcohol dehydrogenase (LADH) in which the active site is specifically metal-depleted [H4Zn(n)2LADH] or specifically Co-**substituted** [Co(c)2-Zn(n)2LADH] have been studied by crystallographic methods. (In these formulae, "n" identifies the noncatalytic zinc ion and "c" identifies the catalytic metal ion.) X-ray data were collected for H4Zn(n)2LADH to 2.7-A resolution and for Co(c)2Zn(n)2LADH to 2.4-A resolution. Difference Fourier maps demonstrate

clearly that the catalytic zinc ions are removed in H4Zn(n)2LADH, whereas the noncatalytic zinc ions are still present. A 2.5-A shift in the sulphur position of **cysteine**-46 and a slight torsion of the imidazole ring of histidine-67 are the only changes in the protein structure that could be detected when compared to the native zinc enzyme. The structure of Co(c)2Zn(n)2LADH is essentially the same as that of the native enzyme. Each cobalt ion is bound to the ligands **cysteine** -46, **cysteine**-174, and histidine-67 and to a water molecule in a distorted tetrahedral geometry. A slight change in the position of histidine-67 was found. No further structural changes could be observed in the protein.

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